

Abstract Book

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Keynote and Plenary Presentations

5th Nordic Metabolomics Conference 2026

PL1

Make metabolomics great again: LC-BinBase with MassWiki

Oliver Fiehn

Introduction

Nontargeted metabolomic annotations are currently performed by subjective criteria and an uncoherent selection of criteria and mass spectral libraries. While some companies do not disclose any metadata to their annotations, even academics may state that 'in-house libraries' were used. MS/MS data of most studies that are published in the literature are not available on public resources such as GNPS, MetabolomicsWorkbench or MetaboLights. At the same time, accurate mass and MS/MS data may often be insufficient to unambiguously annotate compounds from mass spectral libraries.

Methods

MassWiki is a public web resource built on the LC-BinBase database environment, using standardized LC conditions with 43 (HILIC) to 76 (RPLC) internal standards. Users can compare and store (for publication) their MS/MS spectra against all annotations in BinBase, for both identified and unknown compounds. Users who employ the UC Davis standardized chromatography methods (BEH C18 for lipidomics and BEH Amide for HILIC separations), including details of solvent gradients and internal standards, can directly compare their experimental retention times to MassWiki entries for additional confidence. Users can also view, comment on, explore and scrutinize the UC Davis Mass.Wiki entries, opening the door for a transparent and argument-based improvement of metabolomic annotations.

Results

MassWiki currently offers public use and commentaries for six databases (assays), all in positive and negative ESI mode: HILIC-MS/MS for Orbitrap and TripleTOF (QTOF) mass spectra, and RPLC-MS/MS for Orbitrap mass spectra (lipidomics). For public use, all mass spectra can be compared via MassBank of North America (MassBank.us) and GNPS libraries. Internally at UC Davis, compounds are also annotated by licensed libraries including NIST23. The six Mass.Wiki databases currently have 1,500-2,500 annotated compounds per assay, plus up to 10x more unknown compounds. Annotations are based on three parameters: accurate mass of the precursor ions, MS/MS entropy similarity matches, and retention time prediction via a new AI-based metabolomics foundational model, MolRex. MolRex outperforms all other AI- or ML-based algorithms, including our previously published Retip.app SOTA algorithm. All MS/MS spectra, for both unknown and known compounds, are automatically screened against studies and samples at GNPS, MetabolomicsWorkbench and UC Davis. Sunscreen burst diagrams and detailed tables inform users in which types of samples specific samples are found. All mass spectra are also automatically compared to similar spectra within each assay ("Molecular Network Similarity"), similar to GNPS tools. Users can upload 100 raw chromatograms to view Extracted Ion Chromatograms that have matching MS/MS spectra, or view up to 100 randomly chosen raw EIC chromatograms from the UC Davis repository that have matching MS/MS spectra. Mass spectra of unknown (non-annotated) compounds can be investigated by Open Search (same spectra, but different precursor mass), Neutral Loss search (MS/MS spectra with offsets of product ions) and Hybrid Search. Users can leave comments that agree with current annotations ('Likes') or disagreements (with arguments). Overall, Mass.Wiki is open for public comments. We especially invite critical comments on compound annotations to build a trusted nontargeted database as community resource.

PL2

Metrology for Mass Spectrometry Imaging

Josephine Bunch

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Mass spectrometry (MS) is one of the most powerful techniques for chemical analysis and when combined with an imaging modality allows molecules to be visualised in a spatially resolved manner, from the nano- to the macroscale. There are many well established methodologies which can be performed, some under vacuum and others under ambient conditions. There are approaches suitable for analysis and imaging of small and large molecules with performances spanning several orders of achievable spatial resolution. Over the last decade we have observed an increasing appetite for using several techniques in combination. By pursuing a multiscale and multi-omics approach with a range of mass spectrometry imaging (MSI) techniques, a deep understanding of the metabolic deregulation which occurs in many diseases can be obtained. In this presentation, case study examples from the Rosetta Grand Challenge will be used to illustrate the need for metrology in MSI. The presentation will review challenges in determining uncertainty in MSI, and the (daunting) task of establishing a robust quality infrastructure to ensure confidence in data from large scale MSI experiments.

K1

Inside the Tumor and Beyond: NMR-based Metabolic Profiling in Cancer

Tone Frost Bathen

Metabolomics is often presented as a natural fit for the personalized medicine concept, and NMR in particular offers the reproducibility, quantitative accuracy and minimal sample preparation that clinical studies demand — yet clinical implementation remains elusive. Drawing on two decades of work in breast and prostate cancer, I will discuss what metabolic profiling has delivered and what still stands between it and the clinic.

HR-MAS MRS of intact tumor tissue has provided metabolic fingerprints with diagnostic and prognostic value, but has not become a clinical endpoint. I will argue that its lasting contribution is as a research tool that supplies the functional layer genomics lacks and integrates naturally into multi-omics and radiomics pipelines.

Moving from tissue to circulation, serum NMR captures the systemic consequences of cancer treatment — notably shifts in lipoprotein profiles relevant to the elevated cardiovascular risk seen in survivors, and their potential modification through intervention. Characterizing these changes may ultimately point towards new interventions and treatment targets for preventing long-term side-effects in a growing population of cancer survivors.

K2

Towards scalable explainable data mining and matching strategies in untargeted metabolomics

Justin J.J. van der Hooft

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Nature has always been a rich resource of diverse chemistry. Fortunately, in recent years, technological advances in the omics fields have increased our capacity in measuring them. For example, modern mass spectrometers generate information-rich chemical profiles used for untargeted metabolomics studies. Such studies are typical examples of exploratory data analysis, as one does not a priori know what one will discover in the data. However, the current information-rich metabolomics profiles leave researchers with the daunting task to separate valuable signals from everything else. In this keynote, I will highlight our recent contributions in networking and machine learning-based strategies for data mining and matching of metabolite features. These include the work on the SpecReBoot framework that boosts library matching by offering query-focused match scores to rank annotations, and enhances molecular network organization by trimming unwanted spectral links and focusing on more reliable connections. Other key frameworks I will showcase are MS2DeepScore 2.0 that combines positive and negative ionization mode spectra in one supervised model for network or chemical space visualizations, and MS2LDA 2.0 unsupervised substructure discovery to complement spectral library-based annotation by substructure annotations that can be used to create spectral alerts. I will finish the talk with highlighting the important role of Open Science and Software in stimulating community-based science. Altogether, I expect that the presented progress in computational metabolomics tools will empower metabolomics researchers navigating their increasingly complex datasets to find and annotate relevant and novel chemistry from nature or other sources.

K3

Lipidomics studies identify platelet phospholipids that drive coagulation and thrombosis.

Valerie O'Donnell

Blood clotting takes place on the surface of activated platelets and white blood cells through a process requiring the externalisation of aminophospholipids, in particular phosphatidylserine (PS). Between 2008-2016, using lipidomics approaches, we identified and characterised several families of enzymatically oxidized phospholipids (eoxPL) generated through the co-ordinated action of lipoxygenases (LOX) and the Lands Cycle pathway. Large amounts of these are now known to be made by platelets following agonist activation, and they remain cell associated following their formation. In vitro and in vivo studies determined their biochemistry, their cellular biology and their mechanistic role in supporting PS-dependent coagulation. Mice lacking these lipids have a bleeding defect, and form smaller thrombi in vivo as well as being protected against vascular inflammatory disease including aortic aneurysm. Our recent studies have turned to interrogating human vascular inflammation to define whether they play a role in pathological thrombosis. Elevated levels of eoxPL, particularly from platelets, have now been detected in autoimmunity and acute coronary syndrome, as well as in retrieved human arterial thrombi. In mice, inflammatory arthritis leads to higher levels of platelet eoxPL through an interleukin-6 dependent mechanism. Here, they directly drive a pro-coagulant phenotype. Moving forward, we consider whether it's possible to target the pro-coagulant membrane directly to dampen thrombosis in diverse human diseases, alongside direct inhibition of coagulation and platelet aggregation.

K4

From Metabolite Discovery to Mechanism: Decoding Diet-Microbiota–Host Interactions in Early Life

Henrik Munch Roager

Department of Nutrition, Exercise and Sports, University of Copenhagen

The gut microbiota produces a vast array of metabolites whose production is shaped by diet and intestinal factors such as pH and transit time. These metabolites provide an important interface for host–microbial cross-talk, with the potential to influence intestinal barrier function, immune responses, and host development.

During infancy, the gut microbiota is enriched in breastmilk-promoted *Bifidobacterium* species, which are thought to play important roles in early-life development. By combining longitudinal infant cohorts with untargeted metabolomics, we have identified several classes of *Bifidobacterium*-derived metabolites that may mediate host–microbial communication during this critical developmental window, including short-chain fatty acids, aromatic lactic acids, and novel peptides.

In this keynote, I will trace the journey from metabolite discovery in human cohorts to mechanistic validation. I will showcase how metabolomics can be integrated with microbial cultivation, genome mining, and functional experiments to identify the bacterial pathways responsible for metabolite production, uncover host–receptor interactions, and ultimately demonstrate the effects of these metabolites on immune and infant intestinal organoid cells. Together, these studies illustrate how metabolomics can move beyond association to provide mechanistic insights into the molecular dialogue between diet, the developing microbiota and its host.

Gold Sponsor – Bruker

Big Technologies for Small Molecules

Matthew Lewis

Metabolomes are dynamic and multidimensional. High chemical diversity across vast physiological abundance ranges makes comprehensive metabolome coverage a formidable bioanalytical challenge, and one that no single technology can meet alone. Guest appearances from a virtually limitless number of exogenous chemicals from the exposome complicate matters further, requiring an always-open door for discovery. The choice of analytical platform profoundly shapes what we can see, measure, and ultimately discover. Here we present Bruker's uniquely complete multi-technology offering across NMR and high-resolution mass spectrometry, and the scientific insights it enables at speed, depth and scale. The timsMetabo platform, built for 4D-Metabolomics and 4D-Lipidomics with enhanced metabolome coverage, annotation confidence, and data transferability across studies, takes a leap forward in workflow standardization with the introduction of the biocrates MxQuant hybrid workflow. This innovation complements the Avance IVDr NMR platform's own capabilities in merging targeted quantitation and untargeted discovery for uncompromising data generation and biological insight, together capturing the smallest molecules to the largest lipoproteins, ideal for clinical and population-scale metabolomics research. Alongside collaborative insights from Nordic partners, recent innovations in MS and NMR technology are featured. Together, these advancements enable the progressive illumination of the unknown molecular universe and make the metabolic "big picture" that underpins the study of health and disease more accessible.

Gold Sponsor – Thermo Fisher Scientific

Software, hardware and intelligent acquisition strategies for guided unknown metabolite characterization using the Thermo Scientific™ Orbitrap™ Tribrid Apex Mass Spectrometer

Susan S. Bird

The field of metabolomics has come a long way to effectively turn mass spectrometry signals into confident and reproducible chemical identities. As data processing and hardware advances continue to develop, the large chasm of uncharacterized small molecule compounds remains a top pain point for researchers applying global metabolomics strategies. Beyond spectral library matching, which is very effective at identifying known chemical identities, confident unknown annotations require intelligent sample acquisition as well as analysis.

Comprehensive MS/MS strategies such as the AcquireX Deep Scan workflow improve the efficiency of MS/MS collection by excluding background ions detected in blanks and iteratively targeting new relevant precursors in samples of interest across replicate injections. Real-Time Library Search (RTLs) offers a means to run ddMSⁿ experiments with parameters adjusted based on matching compounds to a library. A similarity experiment allows the system to find the closest matching compound in the library in real time, then guide the instrument to collect ddMS³ data on matching fragments to confirm known substructures and non-matching fragments to characterize unknown structures.

In this presentation, we will showcase real-world examples utilizing the intelligently acquired fragmentation as applied to a standard global untargeted experiment. The enhanced workflow is designed to streamline unknown small molecule characterization using intelligently acquired alternative fragmentation and compound discoverer software.

Oral Presentations

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Secretome NMR Profiling of Prostate Tissue Explants and Organoids Reveals Integrated One-Carbon and Nicotinamide Dysregulation

Victoire Bondeville, Juan Carlos Cabellos Guillem, Guro F. Giskeødegård, May-Britt Tessem

Background. The cancer secretome, defined as the ensemble of proteins and metabolites actively released by cells into their surrounding environment, represents an underexplored yet mechanistically informative matrix for cancer metabolic phenotyping. Secretome samples were collected from prostate tissue cultured ex-vivo as explants (cancer, normal, and cancer-adjacent) and organoids derived from these tissues. This study characterized their metabolic profiles using high-resolution magic angle spinning (HR-MAS) ^1H NMR spectroscopy.

Methods. 200 prostate tissue cores, classified as cancer, normal or cancer-adjacent, were obtained from 4 patients undergoing radical prostatectomy and maintained in ex vivo culture. Secretome samples were harvested longitudinally from the explant cultures and from derived organoids, then analyzed by HR-MAS ^1H NMR. Metabolite identification was performed in MATLAB via multiplet analysis and cross-referencing with spectral databases and validated through chemical shift consistency. Metabolite concentrations were expressed relative to blank culture media. Statistical analyses were conducted in R 4.4.

Results. 46 metabolites were identified in the 220 secretomes samples included in this study. Secretome profiles from cancer explants, compared to normal and cancer-adjacent tissues, showed three consistently altered metabolic pathways. In the one-carbon cycle, central in DNA synthesis and epigenetic regulation, trimethylamine, dimethylglycine, and sarcosine were simultaneously detected in tumour-derived samples, indicating heightened one-carbon activity. The polyamine synthesis pathway, critical for cell proliferation [1], was represented by putrescine and spermidine/spermine signals consistent with elevated ornithine decarboxylase activity. A NAD^+ metabolic signature comprising adenine nucleotides, free nicotinamide, and 1-methylnicotinamide (MNA, enzymatic product of nicotinamide N-methyltransferase, NNMT) was identified. Co-detection of nicotinamide and MNA provides direct evidence of active NNMT in the secretome, of particular relevance given that NNMT overexpression drives epigenetic reprogramming in prostate cancer [2]. Cancer explants also exhibited lower citrate than both normal and cancer-adjacent tissues, consistent with established in vivo observations [1] and supporting the biosimilarity of the ex vivo model. **Conclusion.** HR-MAS ^1H NMR profiling of the prostate secretome, encompassing tissue explants and organoids, identifies metabolic signatures distinguishing cancer from both normal and cancer-adjacent tissues: elevated sarcosine and MNA alongside reduced citrate, reflecting dysregulation of the one-carbon cycle, polyamine synthesis, and NAD^+ /NNMT activity. These findings position the secretome as a mechanistically rich and biosimilar matrix for prostate cancer characterization.

[1] Giskeødegård GF., et al. Spermine and Citrate as Metabolic Biomarkers for Assessing Prostate Cancer Aggressiveness. PLOS ONE (2013)

[2] Zhou W, et al. NNMT is overexpressed in prostate cancer and correlates with prolonged PFS and OS. Oncology Letters (2014)

O1-2

Benchmarking Ion Mobility–Resolved LC–MS/MS Spectral Libraries Demonstrates the Need for Instrument-Specific Spectral Libraries

Aninda Mazumdar, Tommaso Stefani, Michaela Plechatá, Mingxun Wang, Zdenek Kamenik

Untargeted metabolomics based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) has become a central approach for comprehensive profiling of small molecules in complex biological systems. By enabling simultaneous detection of thousands of metabolites without prior target selection, LC–MS/MS supports applications ranging from microbiome research and natural product discovery to clinical and environmental studies.

However, reliable metabolite annotation remains a major challenge in untargeted metabolomics, despite the substantial expansion of public LC–MS/MS spectral libraries¹. Current resources still provide limited annotation coverage and often lack collision cross-section (CCS) information, restricting confidence in metabolite annotation. Trapped ion mobility spectrometry coupled with LC–MS/MS (timsTOF HT) addresses this limitation by enabling multidimensional molecular characterization through the integration of accurate mass, retention time, fragmentation spectra, and CCS measurements. Nevertheless, comprehensive ion mobility–resolved spectral libraries covering diverse bioactive compounds and broader chemical space remain scarce, limiting the annotation of detected features in complex biological samples.

We developed an ion mobility–resolved LC–IMS–MS/MS spectral library containing more than 10,000 bioactive compounds spanning diverse chemical classes. Compounds were analyzed using liquid chromatography coupled to a Bruker timsTOF HT platform. Data were processed in MZmine to extract features, generate consensus MS/MS spectra, and assign CCS values to obtain the LC–IMS–MS/MS spectral library². The resulting library was benchmarked against the GNPS2 public spectral database using multiple biological datasets, including gut microbiome fecal samples, microbial culture extracts, and publicly available global datasets acquired on both timsTOF and Orbitrap platforms.

Across datasets, the ion mobility–resolved library increased metabolite annotation rates by 1–10% compared with GNPS2 libraries, with the strongest improvements observed for datasets acquired under similar chromatographic and instrumental conditions. Incorporation of CCS values provided an orthogonal validation parameter that reduced false-positive annotations and improved structural confidence.

This LC–IMS–MS/MS spectral resource substantially expands current metabolomics reference libraries by integrating ion mobility information and increasing chemical coverage. The dataset supports improved metabolite annotation, cross-platform validation, and future AI-driven workflows for metabolite identification and natural product discovery.

1 Bittremieux, W. et al. "The critical role that spectral libraries play in capturing the metabolomics community knowledge." *Metabolomics* 18.12 : 94 (2022).

2 Brungs, C. et al. "MSnLib: efficient generation of open multi-stage fragmentation mass spectral libraries." *Nature Methods*: 1-4 (2025).

O1-3

Acute effects of moderate- and high-intensity exercise on the human milk metabolome.

Marta R. Moksnes, Ankit S. Tanwar, Maëliiss C.C. Lemoine, Emily R. Ashby, Guro F. Giskeødegård, Trine Moholdt

Exercise training regulates systemic metabolism through both acute effects and chronic adaptations across tissues. Postpartum exercise is recommended and might be particularly beneficial for individuals with overweight or obesity. Breastfeeding is likewise recommended for optimal infant growth and long-term health. However, limited knowledge exists regarding the effect of maternal exercise on the human milk metabolome, particularly in lactating individuals with overweight or obesity.

The aim of this randomized crossover trial (NCT number: NCT05745922) was to evaluate the acute effect of moderate- and high-intensity exercise training on the human milk metabolome. We included twenty-seven (n=27) postpartum individuals with BMI > 25 kg/m² who completed three conditions in randomized sequence, separated by 1-week washout periods: 1) High-intensity interval training (HIIT), 2) Moderate intensity continuous training (MICT), 3) Seated rest. Participants completed the first condition on average 52 days postpartum. We collected human milk samples from the participants pre-condition and at 0, 1 and 4 hours post-condition and quantified 422 milk metabolites using untargeted metabolomics profiling (Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS) performed by Metabolon). We used linear mixed models to assess time-by-condition interaction effects for HIIT and MICT across post-condition time points, using rest and pre-condition concentrations as reference points.

We identified significant post-exercise alterations in 45 human milk metabolites compared with rest (p-value < 2.1710⁻³, Principal Component Analysis (PCA) based Bonferroni correction), including replication of previously reported exercise-responsive metabolites (1). Most metabolite changes occurred immediately or 1-hour post-exercise, particularly following HIIT, although several responses were also observed post-MICT. HIIT induced marked increases in citric acid cycle intermediates, including alpha-ketoglutarate, succinate, malate, citrate and aconitate, as well as lactate, whereas these responses were not observed following MICT. In contrast, glucose concentrations were significantly reduced immediately following MICT but not HIIT. Milk metabolites involved in amino acid and nucleotide metabolism were generally increased following exercise irrespective of intensity, with partially overlapping metabolic pathways. Most metabolites returned toward baseline within 4 hours post-exercise; however, alpha-hydroxyisovalerate remained significantly elevated 4 hours after HIIT. Notably, an uncharacterized metabolite increased exclusively at the 4-hour time point following both HIIT and MICT, suggesting potential delayed or sustained metabolic responses. In conclusion, we demonstrate significant acute changes in the milk metabolome post-exercise in postpartum individuals with overweight or obesity.

Lu, C et al. Maternal Physical Activity and Its Relationship to the Human Milk Metabolome and Infant Body Composition. JCEM. 2026 Jan; 111(1):129-138

O2-1

Deep Learning of Serum Metabolomics Identifies Early Multiple Sclerosis Signatures and Reveals No Accelerated Metabolomic Aging at Diagnosis

Jakob Siljebo, Maria Isabel Marulanda Alvarez, Akshai Parakkal Sreenivasan, Aina Vaivade, Asma Al-Grety, Ida Erngren, Henrik Carlsson, Payam Emami Khoonsari, Ingrid Kockum, Anna Karin Hedström, Lars Alfredsson, Tomas Olsson, Ola Spjuth, Joachim Burman, Kim Kultima

Blood-based molecular profiling offers a minimally invasive window into disease biology and biological aging in multiple sclerosis (MS). Liquid chromatography-high-resolution mass spectrometry (LC-HRMS) captures broad metabolic signatures, while deep learning can model complex patterns in high-dimensional data. Although accelerated metabolomic aging has been reported in MS, it remains unclear whether this reflects early disease biology at diagnosis. Here, we evaluated whether serum LC-HRMS profiles distinguish newly diagnosed persons with MS (pwMS) from matched controls (MC) and whether metabolomic age acceleration is present at disease onset. Serum samples from 938 newly diagnosed pwMS and 954 MC in the population-based Epidemiological Investigation of Multiple Sclerosis cohort were analyzed across five LC-HRMS metabolomics assays and transformed into standardized molecular intensity images. A convolutional neural network was trained for MS classification and validated using held-out testing, conformal prediction, and label permutation. Separately, a metabolomic clock model was developed using stacked machine learning, and age acceleration, defined as predicted minus chronological age, was compared between groups using linear mixed models adjusted for chronological age, sex, and matched-pair structure. The MS-status classifier achieved an AUC of 0.99 and an F1 score of 0.96 on the held-out test set. Conformal prediction at 95% confidence achieved 98.2% empirical coverage, with no empty outputs and 9.2% ambiguous outputs, while label permutation reduced performance to chance level (AUC 0.50). The metabolomic clock achieved a root mean square error of 6.73 years. No significant difference in age acceleration was observed between newly diagnosed MS cases and controls ($p=0.515$). These findings show that deep learning applied to serum LC-HRMS data accurately distinguishes newly diagnosed pwMS from MC, indicating that serum molecular profiles capture disease-associated signatures at diagnosis. In contrast, metabolomic age acceleration was not detectable at disease onset, suggesting that aging-related metabolic alterations reported in MS may emerge later in the disease course. Together, this supports blood-based LC-HRMS combined with deep learning as a promising framework for identifying early disease-associated molecular patterns in MS and complementing established approaches to disease characterization.

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Disclosure of interest

Jakob Siljebo: nothing to disclose.

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O2-2

Early serum metabolomics trajectories and severe retinopathy of prematurity in extremely preterm infants: preliminary results from the Mega Donna Mega trial

Mohit Panwar, Payam Emami Khoonsari, Ulrika Sjöbom, Zhongjie Fu, Lois L. E. Smith, Karin Sävman, David Ley, Ann Hellström, **Anders K. Nilsson**

Background. Retinopathy of prematurity (ROP) is a leading cause of childhood blindness, yet metabolic factors underlying disease pathophysiology remain incompletely understood. We used neonatal serum samples from extremely preterm infants in the Mega Donna Mega (MDM) trial to map early postnatal metabolic trajectories in relation to subsequent severe ROP development.

Methods. Untargeted LC–MS/MS metabolomics (positive and negative ESI) was performed on 855 serum samples from 178 extremely preterm infants enrolled in MDM (127 no/mild ROP, 51 severe ROP), collected at up to six time points across postnatal days 0–38. After tiered annotation, 939 features (in-house library + MS2Query exact match) entered the primary analysis. For each feature, we fitted a linear mixed-effects model with a natural cubic spline of postnatal age interacted with ROP severity, adjusting for gestational age, randomized treatment arm in the original trial, and center, with subject random intercepts; empirical Bayes moderation (dream/variancePartition) was complemented by time-resolved contrasts (emmeans) and weighted co-expression network analysis (WGCNA).

Results. Of the 939 features, 31 showed differential longitudinal trajectories between ROP groups at FDR < 0.05. Top hits included glucuronate, theophylline/caffeine, primary and conjugated bile acids, pregnenolone sulfate, urate, hippurate, indole-3-lactate, and several phosphatidylcholines and short-chain acylcarnitines. Time-resolved contrasts indicated that the two groups were indistinguishable on postnatal day 1 but began to diverge around days 4–8, with sustained or widening differences through days 15–28. WGCNA identified four co-expression modules associated with severe ROP: two elevated (bile acid/steroid; gut–liver co-metabolites including glucuronate and urate) and two depleted (glycerophospholipids/sphingomyelins; indole and aromatic amino acid metabolites of likely microbial origin). All four modules were strongly correlated with gestational age.

Conclusion. These preliminary analyses suggest that infants who develop severe ROP carry an early serum metabolic signature, apparent within the first postnatal week, well before clinical ROP is diagnosable. The findings are exploratory, partly confounded by maturity, and require validation in independent cohorts before mechanistic claims can be made.

O2-3

Distinct Metabolic Signatures Distinguish Lung, Colorectal and Ovarian Cancer

Ioanna Tsiara, Efsthia Vouzaxaki, Tobias Sjöblom, Daniel Globisch

Cancer-related mortality remains a global health concern, with lung, colorectal, and ovarian cancers collectively accounting for 30% of worldwide cancer deaths (1). Early diagnosis is critical for survival, yet these cancers often remain asymptomatic until advanced stages, highlighting an urgent need for non-invasive biomarkers. In this study, we performed a comprehensive untargeted mass spectrometry-based metabolomics analysis of 710 plasma samples, including 615 patients with lung (LuCa), colorectal (CRC), or ovarian cancer (OvCa) across disease stages I–IV and 95 non-cancerous controls. To ensure analytical robustness, the cohort was analyzed in three independent batches (n=229, 239, 242) in both positive and negative ionization modes. To ensure data integrity across the three analytical batches, we employed a workflow using SERRF and ComBat for intra- and inter-batch normalization (2). Significant metabolic features were identified through univariate statistical analysis and further evaluated using receiver operating characteristic (ROC) curve analysis to assess their diagnostic potential.

Our investigation identified panels of metabolites associated with lipid and amino acid metabolism, as well as xenobiotic transformation, which showed high discriminatory power. For early-stage (I/II) discrimination between LuCa and CRC, a panel of nine metabolites, including *S*-prenyl-L-cysteine, *S*-propenylcysteine and 3-hydroxypyridine sulfate, achieved an excellent combined ROC AUC of 0.95. In late-stage (III/IV) comparisons, a distinct nine-metabolite panel of phenol sulfate, uridine and benzoic acid achieved an AUC of 0.89. Furthermore, a six-metabolite panel, including epipregnanolone sulfate and several acylcarnitines, effectively differentiated early and late-stage OvCa (AUC = 0.79). |

While shared metabolic features across cancer types suggest common pathways of stress and inflammation, cancer-specific markers reflect unique tissue biology and dietary or microbiome influences. Our findings demonstrate that plasma metabolite panels can accurately distinguish between cancer types and disease stages. This pan-cancer biological framework provides a basis for integrating metabolic profiling with genomic and microbial data in future validation studies to enhance early detection and clinical monitoring. |

1. Bray F, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2024 May-Jun;74(3):229-263. |
2. Fan S, et al. Systematic Error Removal Using Random Forest for Normalizing Large-Scale Untargeted Lipidomics Data. *Anal Chem*. 2019 Mar 5;91(5):3590-3596. |
3. Tsiara I, et al. Distinct Metabolic Signatures Distinguish Lung, Colorectal and Ovarian Cancer. *medRxiv*. 2026 Apr 8; doi: 10.64898/2026.04.08.26350309. |

Optimised Extraction of Dried Blood Spots for Untargeted HILIC Metabolomics: A Factorial Comparison with Independent Confirmation

Étienne Ljóni Poisson, Freyr Jóhannsson, Jón Jóhannes Jónsson, Óttar Rolfsson

Untargeted metabolomics of dried blood spots (DBS) holds transformative potential for clinical diagnostics, newborn screening, and rare disease biomarker discovery. However, clinical translation is currently impeded by a lack of systematically validated, matrix-optimised analytical pipelines. We describe the development and characterisation of a UPLC-QTOF platform integrating hydrophilic interaction liquid chromatography (HILIC) in both positive and negative electrospray ionisation modes. This pipeline is anchored by a 13-component isotopically labelled internal standard cocktail and a 387-compound spectral library of clinically relevant small molecules.

To define the optimal extraction protocol, a factorial comparison evaluated four solvent systems, two post-processing strategies (direct supernatant versus vacuum-dried reconstitution), and two extraction durations (45 versus 90 minutes) using 3.2 mm DBS punches. Buffer composition was the overwhelmingly dominant variable influencing metabolite coverage and signal intensity. Aqueous methanol systems substantially outperformed 100% methanol, the current newborn screening reference standard, for the recovery of highly polar, multiply-charged species such as nucleotides and phosphorylated sugars.

The 45-minute direct supernatant protocol using 4:1 methanol-water achieved optimal analytical performance, recovering 24.8% of negative and 24.4% of positive tracked library entries with good within-group reproducibility (~10% CV). Conversely, vacuum-drying and pellet reconstitution systematically reduced both coverage and precision. A targeted follow-up experiment confirmed that this evaporative and adsorptive loss could not be rescued by optimising the downstream reconstitution solvent. Furthermore, an experiment testing sequential solvent addition order yielded modest precision gains but identical overall metabolite coverage compared to premixed buffers.

In conclusion, aqueous methanol direct supernatant extraction fundamentally outperforms standard reconstituted pellet protocols for untargeted HILIC analysis of dried blood spots. Eliminating the drying step removes a critical point of variance and prevents the irreversible loss of labile or adsorption-prone species. This systematically validated workflow currently underpins a prospective 1,000-newborn metabolic baseline cohort and a rare disease biomarker discovery programme in Iceland.

O2-5

Combining spatial metabolomics and transcriptomics to understand long term changes in the brain following a TBI

Dominika Olesova, Ilia Evstafev, Daniel Anthony, Matej Oresic, **Alex Dickens**

Traumatic brain injury (TBI) is a leading cause of injury-related death and disability worldwide, affecting an estimated 64–74 million people each year. Although most individuals with mild TBI recover within weeks, approximately 15–30% experience persistent cognitive, behavioural, and physical symptoms that can last months or even years. Increasingly, TBI is therefore recognised as a chronic condition, with long-term neurological dysfunction thought to be partly driven by a post-injury metabolic “energy crisis.” Understanding the cellular and metabolic changes that occur after injury is essential for identifying mechanisms that contribute to long-term pathology. In this study, we investigated spatially resolved molecular changes in the mouse brain following mild TBI.

A controlled cortical impact (CCI) model was used to induce mild TBI in the left cortex of mice. Injury was delivered using a linear impactor following craniotomy. Animals were sacrificed at 24 hours and three weeks post-injury. Spatial metabolomics were performed using a MALDI-2 timsTOFflex mass spectrometry platform. Spatial transcriptomics was conducted on sequential tissue sections using the 10x Genomics Visium platform.

Despite the injury being classified as mild, behavioural testing revealed anxiety-like behaviour persisting up to three weeks after injury, indicating lasting functional effects. Spatial transcriptomic analysis demonstrated continued immune activity within the brain at this time point. Much of this response was associated with GFAP-expressing astrocytes, alongside evidence of sustained microglial reactivity at the injury site. Spatial lipidomics revealed distinct accumulation patterns of ceramide lipids in the three-week samples, consistent with the presence of fibrotic astrocytes. In contrast, macrophage-associated regions showed increased levels of diacylglycerols and triacylglycerols.

By integrating multiple spatial omics layers, we can resolve cell-type-specific metabolic changes within injured brain tissue. This approach provides mechanistic insight into the metabolic responses following TBI and highlights potential pathways that may represent targets for future therapeutic development.

O2-6

Longitudinal metabolomic trajectories across the development of Inflammatory Bowel Disease

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Background: Inflammatory bowel disease (IBD) is an immune-mediated disease leading to chronic inflammation in the gastrointestinal tract. Elevation of inflammatory lipids, including glycerophosphocolines, eicosanoids and fatty acids and conjugates, have been detected up to 14 years before diagnosis¹, but whether these lipids remain elevated across IBD development remains unknown. The aim was therefore to characterize trajectories of IBD-related metabolites over time and identify metabolites at specific timepoints that could be informative for disease prognosis.

Methods: Utilizing data from the Danish National Biobank linked to Danish national health registers, we identified patients with IBD who had available samples from the preclinical phase, around diagnosis and the post-diagnostic phase. We conducted untargeted metabolomics using liquid chromatography-mass spectrometry to identify changes of 432 metabolites from 46 metabolic classes, which had all shown alternations in the pre-diagnostic phase in a previous analysis of the data¹. Metabolite trajectories were investigated using linear mixed models.

Results: Levels of 2 and 22 metabolites were significantly changed from the preclinical phase to diagnosis, and from diagnosis to the post-diagnostic phase, respectively ($FDR \leq 0.05$) with 101 and 139 metabolites nominally changed ($P \leq 0.05$). All metabolites previously associated with increased risk of IBD¹ were increasing during the preclinical phase until diagnosis. Stratifying preclinical samples into early and late sampling revealed no significant associations in the late preclinical phase, whereas 95 metabolites were significantly increased from early preclinical phase to around diagnosis. This indicates that metabolites that increase do so already in the early preclinical phase (4-14 years before diagnosis). A similar trend occurred in the post-diagnostic phase, where late post-diagnostic samples were decreased more compared to samples closer to diagnosis. Glycerophosphocolines were enriched among significant associations in the early preclinical ($P = 3.09 \times 10^{-9}$) and in the post-diagnostic ($P = 1.67 \times 10^{-3}$) phases. Interestingly, eicosanoids and fatty acids and conjugates remained unchanged throughout disease progression. These findings imply a possible role of phospholipids and their inflammatory mediators in IBD, starting already in the early preclinical phase.

Conclusion: This is the first study to model metabolite trajectories across development of IBD. Levels of glycerophosphocolines, elevated in preclinical cases compared to controls¹, were also increasing from the early preclinical phase to around diagnosis, after which they normalize to preclinical levels. As lipid classes are implicated in both IBD progression and post-diagnosis, future studies should utilize lipidomics to clarify the causal pathway in disease and remission.

Lemser et al. (2026). Clinical Gastroenterology and Hepatology. S1542356526001370.

Preferred metabolomics session:

Session 1: Clinical Metabolomics

O2-7

Changes in serum lipids during breast cancer treatment with aromatase inhibitors- results from a randomized controlled trial

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Introduction

Cancer treatment may bring about late effects, including an increased risk of cardiovascular disease (CVD) in breast cancer survivors. Investigating the systemic effects of cancer treatments may help explain this effect and to identify measures for prevention of negative effects. The aromatase inhibitors letrozole and exemestane are widely used to treat estrogen receptor (ER) positive breast cancer in postmenopausal patients. In this study, we investigated changes in serum lipid and metabolic profiles during neoadjuvant treatment with letrozole and exemestane, and examined the association of these changes with treatment response.

Methods

Postmenopausal patients (n=99) with ER-positive, HER-2 negative, and locally advanced breast cancer were randomized to treatment starting with either letrozole or exemestane for at least 2 months followed by another 2 months on the alternative treatment. Serum samples were collected at three different timepoints: 1) before treatment; 2) after 2 months of treatment (still on the first drug); 3) after 4 months of treatment (while still on second drug). Steroid hormones were quantified by an LC-MS/MS-based method and NMR spectroscopy was performed to determine serum lipoprotein subfractions, small-molecular metabolites, and inflammation markers. The longitudinal data were analyzed by linear mixed models and repeated measures ASCA+ (RM-ASCA+) analysis.

Results

The aromatase inhibitors had similar effects on steroid hormones, and as expected, both aromatase inhibitors significantly decreased serum levels of estrone and estradiol. Clear changes in lipoprotein subfractions were evident during treatment with exemestane, with pronounced decreases of phospholipids and cholesterol of the largest high-density lipoproteins (HDLs). These patterns were not evident during letrozole therapy. Interestingly, the inflammation markers GlycA and GlycB were significantly higher before treatment in patients with a poor response to treatment (>50 % residual tumor) compared to patients with a good response (<50% residual tumor) ($p < 0.02$).

Conclusion

Broad characterization of serum metabolism provides insight into the metabolic effects of cancer treatment and may reveal mechanisms for treatment resistance and long-term effects.

O3-1

AutoDIP: An Automated Direct Infusion Platform for High-Throughput Metabolomics

Lucie Davidová and Ingela Lanekoff

Direct infusion mass spectrometry (DI-MS) enables rapid analysis and is therefore increasingly attractive for metabolomics studies and clinical applications. However, broader implementation of large-scale DI-MS workflows remains limited by several challenges, including low reproducibility, carryover, solvent evaporation from low-volume samples during extended acquisition sequences, and the need for continuous operator supervision. Moreover, dedicated software tools tailored for direct infusion datasets remain scarce. Here, we present AutoDIP, a fully automated end-to-end direct infusion platform designed for robust, high-throughput metabolomics of low-volume biological samples.

The AutoDIP platform integrates a motorized XYZ stage with a custom LabVIEW-controlled interface enabling automated sampling from predefined multiwell substrates using a direct infusion probe (DIP). The DIP consisted of a stainless-steel capillary (350 μm OD, 50 μm ID, 7 cm length) operated using nebulizing N₂ gas (8,000 mbar) and 2.3 kV spray voltage for self-aspiration due to the Venturi effect. The platform was implemented for automated analysis of up to 20-50 μL samples from a 192-well plates and coupled to an Orbitrap IQ-X mass spectrometer operated at 500,000 resolution (at m/z 200). Optimal sampling time was established to be 20 s per well, resulting in inter-well variability below 15%, while automated wash cycles reduced carryover to below 5%. Aluminum sealing minimized solvent evaporation during extended acquisition sequences. In addition, dedicated software was developed for processing of continuous DI-MS datasets, including peak extraction, alignment, normalization, blank subtraction, filtering, and visualization.

The platform was validated using metabolite standards, as well as INS-1 cell extracts. Subsequently, AutoDIP was applied to investigate metabolic adaptations in boundary cap neural stem cells exposed to space microgravity during a 25-day International Space Station mission. Despite highly limited sample volumes ($\sim 30 \mu\text{L}$), clear metabolic separation between space-exposed and control cells was observed. Space-exposed cells showed increased levels of acylcarnitines, glucose and several amino acids, alongside decreased phospholipid abundance, suggesting shifts in energy metabolism and membrane-associated processes following microgravity exposure.

Overall, AutoDIP establishes a scalable and reproducible automated DI-MS workflow for high-throughput metabolomics of limited-volume biological samples and demonstrates strong potential for applications where sample amount and analytical throughput are limiting factors.

O3-2

A Large-Scale LC-MS Approach to Identify Conjugated Bile Acids in Human Fecal Samples Using Untargeted Tandem Mass Spectrometry

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Many studies provide evidence suggesting gut microbe-derived lipids are crucial in host metabolism. Gut microbes can form novel conjugated bile acids in the intestine, and increasing evidence suggests they influence immune development in early life, and dysregulation has been linked to the onset of type 1 diabetes. Previous untargeted bile acid approaches to study conjugated bile acids have been limited by the lack of authentic standards and the similar fragmentation behavior of isomeric analytes. By exploiting combinatorially synthesized standard mixtures and reference spectral libraries, we developed a targeted workflow encompassing 105 bile-acid amino acid conjugates and applied it to large-scale analyses of thousands of fecal samples from a longitudinal birth cohort study and an adult cohort. Prior to biological sample batches, the LC and MS conditions were optimized to enhance detection and MS2 acquisition. Analysis time was reduced by half using dual-column LC equipment compared to the single-column method and without compromising chromatographic separation. A high-confidence annotation level was achieved by matching retention time, precursor mass and fragmentation spectra in standard mixtures and samples. Our method development revealed several previously unrecognized isomers formed during the combinatorial standard procedure and detected in fecal samples. For example, two distinct species for UDCA-Lysine were resolved chromatographically, and two distinct species for UDCA-Proline were resolved in the ion mobility dimension. This study highlights the importance of method development to enable reliable analysis of novel analytes with high confidence in biological samples and demonstrates the use of combinatorial chemistry to help with resolving the mechanistic roles of these analytes.

O3-3

Knowledge-guided analysis of postprandial metabolomics data using coupled tensor factorizations

Gaute Johannessen, Geert Roelof van der Ploeg, Evrim Acar

Understanding human metabolic responses to diet is crucial for characterizing cardiometabolic phenotypes and advancing precision health. Time-resolved metabolomics measurements can naturally be arranged as subject by metabolites by time tensors [1]. Tensor factorizations offer a powerful approach for interpretable pattern discovery from such multiway arrays, simultaneously revealing subject stratifications, metabolite clusters, and temporal response profiles. However, metabolomics measurements are noisy with high individual variation. Recently, it was shown that guiding real metabolomics analysis with simulated data from a human metabolic model improves pattern discovery through coupled tensor factorizations [2]. However, this approach was limited to the subset of metabolites available in both datasets and was only evaluated using T0-corrected (baseline-corrected) preprocessing.

In this paper, we apply coupled tensor factorizations with linear coupling in the metabolites mode [3,4] to enable all metabolites to be included in the joint analysis. Plasma metabolomics data of the COPSAC2000 meal challenge test (133 subjects by 161 metabolites by 8 time points) and simulated challenge data from a human whole-body metabolic model [5] (50 virtual subjects by 6 metabolites by 8 time points) were arranged as tensors. In the joint analysis, we consider two tensor models for the real data: CP, which assumes shared temporal profiles across subjects, and PARAFAC2, which allows subject-specific time profiles. For the simulated data we use the CP model. We assess model performance using correlations of the extracted patterns with body composition and insulin resistance measures.

Our results demonstrate that joint analysis of real and simulated data improves the pattern discovery performance, in particular, when both datasets are jointly analyzed using coupled CP models with linear coupling (with or without T0-correction). This is due to better recovery of an insulin/glucose-related component by guiding the analysis of noisy real data with relatively clean simulated data.

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O4-1

Plasma multi-omics profiles linking body composition with the risks of diabetes and cardiovascular disease in adult women.

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Objectives: Body Composition is a critical driver of cardiometabolic risk, but the underlying molecular pathways linking the association between the two are not fully understood. We characterized molecular phenotype signatures reflecting body composition and examined their mediation role in the association with the risks of type 2 diabetes (T2D) and cardiovascular disease (CVD). **Methods:** Participants were 4,750 women from the Swedish Mammography Cohort-Clinical. Untargeted metabolomics and targeted proteomics profiles were analyzed in fasting plasma samples at baseline. Random forest models were performed to identify the combination of the most relevant metabolite and protein biomarkers related to baseline total and regional body fat (android and gynoid fat) and anthropometric indices (body mass index (BMI) and fat mass index (FMI)). Overall adiposity was calculated using a principal component analysis by integrating all individual body composition measures. Multivariate Cox models were used to assess the prospective association of body composition measures and the risk of T2D and CVD. Causal mediation analysis was conducted to assess the proportion of mediation of the identified metabolites and proteins in the body composition and T2D and CVD associations. **Results:** A multi-omics profile comprising 19 metabolites and 24 proteins was significantly associated in common with all body composition measurements. Annotated metabolites included glycerophospholipids, sphingolipids, fatty acyls, glycerolipids, bile acids, and hexoses, while proteins were involved in the inflammatory response, signal transduction, cholesterol homeostasis, and regulation of appetite and energy balance. There was a linear positive association between overall and individual body composition measures and T2D and CVD risk. The proportions mediated by the multi-omics profile for T2D was 70–90% and for CVD 60–70%. **Conclusion:** We identified a multi-omics profile associated to body composition and demonstrated the mediation role of this profile in the body composition and T2D and CVD risk associations. Our findings offer new insights into the underlying mechanisms of the body composition and cardiometabolic risk relationship, which is critical for improved cardiometabolic disease prevention.

O4-2

Sex-specific lipid-mediated mechanisms drive MASLD progression revealed by paired liver–blood multi-omics

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common chronic liver disease worldwide and displays marked biological heterogeneity and sexual dimorphism. However, the molecular mechanisms driving disease progression remain incompletely understood. Here, we performed an integrative multi-omics analysis of paired liver tissue and serum samples from 211 biopsy-confirmed, morbidly obese individuals with MASLD undergoing bariatric surgery. Hepatic transcriptomics, metabolomics, and lipidomics were integrated with serum metabolomics to define compartment-specific and sex-dependent molecular networks associated with steatosis, inflammation, and fibrosis.

Across sexes, MASLD was characterized by coordinated suppression of hepatic amino acid metabolism and extensive lipid remodeling. Liver lipidomics revealed accumulation of triacylglycerols together with depletion of membrane lipids, including phosphatidylcholines, sphingomyelins, and ceramides. Transcriptomic analyses showed downregulation of amino acid metabolic pathways alongside activation of inflammatory and extracellular matrix programs. In contrast, circulating metabolites frequently displayed inverse patterns relative to liver tissue, particularly for amino acid and bile acid–related metabolites, which were decreased in liver but elevated in serum, indicating compartment-specific metabolic regulation and systemic metabolite spillover.

Sex-stratified analyses revealed marked differences in disease architecture. In men, MASLD progression was dominated by a focused triacylglycerol-centric pathway strongly associated with steatosis and inflammation. In women, disease progression involved broader and highly interconnected networks linking lipid metabolism, amino acid catabolism, and immune signaling pathways. Lipid modules enriched in phosphatidylcholine and phosphatidylethanolamine species were closely associated with amino acid metabolic modules and transcriptomic programs, including PPAR signaling.

Mediation analyses identified hepatic lipid remodeling as a central mechanism linking transcriptional programs to histopathological outcomes. In men, a single triacylglycerol/diacylglycerol module mediated most transcriptional effects on steatosis and inflammation, whereas women exhibited distributed mediation across multiple lipid classes. Together, these findings demonstrate that MASLD progression is driven by sex-specific lipid-mediated molecular networks that cannot be inferred from circulating biomarkers alone, supporting tissue-informed and sex-specific precision medicine approaches.

O4-3

HRMS-based multi-omics integration strategy to map aging in *Caenorhabditis elegans*

Klidel Fae Rellin, George Rosenberger, Nikolas Kessler, Aiko Barsch, **Ivo Kwee**, Murat Akhmedov, Ann-Christine König, Stefanie Hauck, Matthew R. Lewis, Michael Witting

Understanding the molecular basis of ageing requires more than cataloguing individual biomolecules — it demands a systems-level view of how metabolites, lipids, and proteins act in concert. *Caenorhabditis elegans* offers an ideal platform for this: its short lifespan, genetic tractability, and well-characterized longevity mutants make it a cornerstone of ageing research. Yet despite decades of omics profiling in this organism, most studies remain siloed within a single analytical domain. We present a harmonized HRMS-based workflow that synchronizes proteomics, metabolomics, and lipidomics acquisition and processing into a single integrative framework, applied here to dissect the molecular phenotype of the long-lived *daf-2* insulin/IGF-1 receptor mutant.

Wild-type N2 and *daf-2* (e1370) worms were grown and harvested under identical conditions, with all three omics layers derived from the same biological extracts across five replicates. Proteomics was acquired via DIA-PASEF on a timsTOF HT and processed in Spectronaut® (v20), while metabolomics and lipidomics used 4D-MoRE (timsMetabo) and DDA-PASEF (timsTOF HT) acquisition modes processed in MetaboScape® (v2026b). Downstream integration was performed in Omics Playground using multi-omics WGCNA, MOFA, LASAGNA, and SNF to extract cross-domain regulatory structure.

The integrated dataset captures a coherent and internally consistent picture of metabolic reprogramming. In *daf-2* mutants, energy metabolism shifts away from biosynthetic growth: glycolytic intermediates and nucleotide precursors decline, while TCA cycle activity and mitochondrial fatty acid oxidation intensify, evidenced by accumulating acyl-carnitines and organic acids. Lipid remodeling reinforces this shift with triglycerides accumulate with longer, more unsaturated acyl chains, and phospholipid class ratios change in ways consistent with membrane stress adaptation and a dauer-like physiological state.

At the protein level, this metabolic rewiring is reflected in coordinated upregulation of stress defense, detoxification, and innate immunity machinery, alongside suppression of vitellogenins and reproduction-linked proteins which is a hallmark of DAF-16/FOXO transcriptional activation. These signals did not merely co-occur across layers: network integration identified insulin/IGF-1 signaling as the primary axis organizing variation across all three omics domains simultaneously.

The consistency observed across omics layers affirms both the canonical *daf-2* biology and the analytical strategy underlying it; underscoring that cross-domain interpretability is contingent on harmonized sample preparation, acquisition, and scale-aware normalization from the outset.

Metabolite-proteome interactions are pleiotropic disease drivers in kidney disease

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Kidney tubular dysfunction is a hallmark of kidney injury, yet the underlying mechanisms remain incompletely understood. When kidneys fail, tubules cannot secrete gut-derived metabolites, some of which are toxic. In this study, we aimed to identify these gut-derived metabolites that accumulate in the kidney and understand their internal protein targets. To this end, we applied a multi-omics approach using metabolomics, proteomics, and thermal proteome profiling (TPP) across rodent kidney injury models induced by cisplatin, adenine, and ischemia-reperfusion injury (IRI). Integrated data from proteomics and TPP experiments of rodent kidney tissue revealed that protein expression and conformational changes are regulated differently in injury. By profiling four mouse models of kidney injury alongside model-specific patterns of metabolite secretion, we identified three metabolites that accumulate in tubular compartments, likely due to disrupted active transport (3-indoxyl sulfate (3IS), indole-3-lactic acid (ILA), and N1-Methyl-2-pyridone-5-carboxamide (2PY)). Proteomics supported metabolite accumulation, revealing that 45 transporters were downregulated across kidney injury models, including Slc22a8, Slc22a6, and Abcc2, which mediate renal excretion of toxic metabolites. Proteomic profiling of laser-captured proximal tubules across injury models revealed that the only protein commonly regulated across models was Nqo1, involved in quinone detoxification. Phosphoproteomics revealed that a cyclin-dependent kinase (CDK) motif is overrepresented across kidney injury models, although this is not driven by toxic metabolites, as confirmed by kinome profiling. To elucidate the role of gut-derived metabolites, we used thermal proteome profiling, and we found that these metabolites functionally engage with transporters and mitochondrial proteins. In particular, 3IS impaired the function of key electron transport chain components, especially complex I. Consistent with this, native tubule microfluidics revealed that the supply of 3IS enhances riboflavin release. This points to a cofactor-linked mechanism involving mitochondrial complexes I/II, which depend on two riboflavin-derived molecules — flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Both are essential for ATP production and for the proper function of the detoxification enzyme Nqo1 in renal cells. Imposing additional metabolic stress via aromatic amino acid loading rapidly perturbed complex I function through 3IS, as demonstrated by changes in tricarboxylic acid cycle metabolite turnover in native tubules, particularly the malate pool. Together, these findings indicate that kidney injury induces protein conformation remodeling, that propagates into metabolic alterations, and that accumulated metabolites can further modulate injury through direct metabolite–proteome interactions, potentially amplifying renal dysfunction.

O5-1

Steroid hormones as mediators of endocrine disruptions related to cannabis consumption in young men

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Steroid hormones are crucial metabolites for male reproductive function. Their interplay with exogenous environmental chemicals is the focus of current research on the global decline in fertility in Western countries. Due to the expression of cannabinoid receptors throughout the hypothalamic–pituitary–gonadal (HPG) axis, the consumption of marijuana has been hypothesized to alter endocrine and reproductive function in men, via the action of steroid hormones.

To investigate this hypothesis, the main endogenous steroids were quantified in serum samples from 94 young Swiss men (18-23 y.o.), using LC-MS/MS with an innovative one-point internal calibration approach 1. Half of the participants declared cannabis use and showed detectable levels of the corresponding biomarkers (THC and THC-COOH).

While previous reports from the literature showed inconsistent findings regarding the effect of cannabis use on the sole testosterone levels, our study confirmed increased concentrations of all bioactive testicular androgens. Indeed, androstenedione ($p=0.008$), testosterone ($p=0.002$), and dihydrotestosterone ($p=0.029$) were significantly increased in cannabis users compared to non-users 2.

Our steroid profiling was then extended to 171 compounds in a multi-targeted metabolomics strategy 1.

Further investigation of androgen metabolism revealed that the C11-oxy derivatives, which are produced specifically in the adrenal, were not affected by cannabis usage, contrary to their gonadal counterparts. Therefore, intake of exogenous cannabinoids might selectively affect testicular androgen synthesis without altering adrenal or peripheral pathways, possibly via direct effects on the testes, or disruption of hypothalamic–pituitary–gonadal (HPG) axis function.

Such hypotheses from this cross-sectional study would need to be confirmed with mechanistic experiments. However, relevant models such as human Leydig cells are currently not available.

In the extended steroid profile, two progesterone metabolites, 11 β -hydroxyprogesterone (11 β -OHP4) and 5 β -dihydroprogesterone (5 β -DHP4), displayed the most striking increase in cannabis users ($p<0.0001$). The latter group could be further stratified using THC and THC-COOH as biomarkers of chronic consumption. Thus, thanks to both univariate and multivariate analyses (PLS and OPLS-DA), we were able to identify 11 β -OHP4 as a binary biomarker of cannabis use versus non-use, whereas 5 β -DHP4 could be linearly related to the intensity of cannabis smoking with a dose-dependent relationship.

Altogether, these results demonstrate the crucial importance of extending steroid profiling to hundreds of individual metabolites to characterize systemic perturbations of the steroidome while simultaneously relating them to specific, local alterations of hormonal biosynthesis.

O5-2

Glutaminolysis Promotes Inflammation in White Adipose Tissue

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Obesity is characterized by chronic low-grade inflammation in white adipose tissue (WAT). We have previously demonstrated that glutaminolysis is enhanced in obese WAT and stimulated by TNF- α in mature adipocytes. Here, we investigated whether TNF- α -driven glutaminolysis sustains inflammatory signaling through metabolic and epigenetic remodeling in adipocytes. Human adipocytes were stimulated with TNF- α and subjected to pharmacological inhibition or genetic depletion of glutaminase (GLS). Isotope-tracing fluxomics, mitochondrial bioenergetics, and ChIP-seq analyses were performed to dissect glutaminolysis-dependent mechanisms, using CCL2 expression and secretion as inflammatory readouts.

TNF- α reduced glucose uptake, glycolysis, lipogenesis, and ^{13}C -glucose incorporation into glycolytic and TCA cycle intermediates, while increasing OCR and enhanced glutamine anaplerosis. TNF- α also altered the subcellular localization of the metabolic enzyme ATP-citrate lyase (ACLY), promoting its nuclear accumulation and increasing H3K27 acetylation at inflammatory gene loci. Inhibition of glutaminolysis attenuated TNF- α -induced CCL2 expression and secretion and decreased H3K27 acetylation at inflammatory gene loci, including the CCL2 locus, demonstrating that glutamine metabolism is required to sustain inflammatory signaling through epigenetic regulation of inflammatory gene expression.

Together, these findings demonstrate that TNF- α induces broad metabolic rewiring in adipocytes, shifting cells away from glucose-supported anabolic metabolism toward glutaminolysis-dependent mitochondrial and nuclear signaling pathways that sustain inflammation. Targeting GLS or ACLY attenuated adipose tissue inflammation, identifying glutaminolysis as a potential therapeutic target in obesity-associated WAT inflammation.

O5-3

Targeted Spatial Metabolomics for Mapping Lipid Mediator-Related Inflammation in the Lung

C.E. Wheelock, M.J. Smith, J. S  fholm, A. Firsova, C. Samakovlis

Introduction

Asthma is characterized by chronic inflammation in the lung that is mediated by a combination of immune and structural cells. These different cell populations synthesize lipid mediators (e.g., eicosanoids, octadecanoids), which impact asthma pathogenesis via controlled regulation of inflammatory processes. There is a need for methodology to determine the spatial heterogeneity of lipid mediator metabolism and the associated cell types in the lung. We present here a multi-modal method to perform combined targeted mass spectrometry imaging (MSI) and targeted spatial transcriptomics in lung tissue.

Methods

Distal lung explants were obtained post-excision from patients undergoing lobectomies and precision cut lung slices (PCLS) were prepared using a Compresstome  . PCLS were then functionally challenged ex vivo (e.g., anti-IgE, IL-13). Female C57BL/6 mice were sensitized intranasally with house dust mite HDM (1   g) followed by challenge (10   g). Mice were then sacrificed and lungs excised. To spatially localize lipid mediator production, tissue was embedded in carboxymethylcellulose (CMC), cryo-sectioned and metabolite distribution was mapped using desorption electrospray ionization (DESI) coupled to targeted multiple-reaction-monitoring (MRM) (DESI-MRM) using a Xevo-TQ Absolute triple quadrupole. Single Cell Resolution IN Situ Hybridization On Tissues (SCRINSHOT) was subsequently conducted on the same cryosection to map RNA and profile cellular niches.

Results

Spatial eicosanoid mapping revealed highly structured distributions in HDM-challenged lungs. Products of cyclooxygenase (COX), including prostaglandin E2 (PGE₂) and 11-hydroxyeicosatrienoic acid (11-HETE), showed broad parenchymal distribution with airway enrichment, were strongly correlated (Spearman's $\rho=0.553$, bivariate Moran's $I=0.391$), and together were significantly elevated in airways post-challenge ($p<10^{-13}$). Conversely, the lipoxygenase (LOX) product 11-hydroxyeicosatrienoic acid (12-HETE) displayed a distinct spatial pattern with weaker PGE₂ correlation (Spearman's $\rho=0.393$, bivariate Moran's $I=0.290$), indicating LOX-mediated formation in separate tissue microenvironments. 12-HETE displayed a localized increase in airway inflammation following challenge ($p<10^{-19}$), which aligns to previous studies reporting an increase upon HDM challenge. mRNA was stable post-DESI-MRM acquisition up to 12 hours, enabling subsequent SCRINSHOT analysis. This multi-modal workflow was able to sequentially co-register metabolites and mRNA distributions from the same cryosection, enabling visualization of metabolic and cellular responses to ex vivo challenges. Results were integrated with histological staining to correlate metabolite and cellular patterns with physiological responses.

Conclusions

This targeted multimodal integration enables routine visualization of the distribution of metabolites and associated cell type in the lung. These pathway-specific spatial distributions are indistinguishable by bulk analysis, demonstrating the power of spatial metabolomics to resolve compartmentalized eicosanoid biosynthesis during allergic airway inflammation.

O6-1

PFAS and OH-PCB Exposure is Associated with an Altered Bile Acid Homeostasis in Multiple Sclerosis

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Background: Persistent environmental contaminants such as per- and polyfluoroalkyl substances (PFAS) and hydroxylated polychlorinated biphenyls (OH-PCBs) are immunomodulatory compounds that may interact with metabolic pathways relevant to autoimmune disease. Cholesterol is a precursor of bile acids (BAs), steroid hormones, and vitamin D metabolites, which are involved in immune regulation, endocrine signaling, and central nervous system function. BAs regulate gut-liver-immune signaling and are altered in multiple sclerosis (MS). However, it remains unknown whether environmental exposures contribute to the BA dysregulation observed in MS.

Methods: In this population-based study, we analyzed serum concentrations of 17 PFAS, 3 OH-PCBs, cholesterol, 24 BAs, and an additional 15 cholesterol-derived metabolites. The study included 907 people with MS (pwMS) and 907 matched controls (MC) from the Epidemiological Investigation of Multiple Sclerosis (EIMS) cohort. We assessed associations between individual contaminants and cholesterol and its derived metabolites, including BAs, using linear mixed-effect regression models with interaction terms between environmental exposure and MS case-control status, and between environmental exposure and sex. We evaluated co-exposure effects using quantile g-computation. Models were adjusted for demographic and lifestyle covariates.

Results: Long-chain PFAS and OH-PCBs were associated with increased circulating cholesterol levels and decreased concentrations of the BA intermediate 7 α -hydroxy-4-cholesten-3-one, with more pronounced effects in females. Associations with the steroid hormone pathway were modest and inconsistent, with no MS-specific patterns detected. Long-chain PFAS were associated with decreased concentrations of secondary non-conjugated BAs in MC and pwMS, with consistently more pronounced effects in females with MS. Moreover, in pwMS both PFAS and OH-PCBs were associated with increased concentrations of several secondary conjugated BAs. Notably, glycolithocholic acid sulfate (GLCA-S), a BA previously linked to disability worsening, was elevated across multiple exposure classes in pwMS but not in MC. Mixture analyses reinforced these findings, demonstrating more pronounced and complex pollutant-BA associations in pwMS, especially involving secondary BA pathways.

Conclusions: PFAS and OH-PCB exposure were associated with MS-specific alterations in BA metabolism in pwMS, characterized by reduced non-conjugated and increased secondary conjugated BAs. Together with concurrent alterations in upstream cholesterol metabolism, these findings suggest that persistent environmental contaminant exposure may differentially influence cholesterol partitioning and gut-liver axis function in pwMS compared to controls, potentially contributing to pathways relevant to MS progression.

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O6-2

Exploring human-associated *Streptomyces* as a source of novel bioactive metabolites using untargeted metabolomics

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The emergence of antifungal resistance represents a growing global health concern, while the discovery of new antifungal agents remains limited. Natural products produced by Actinomycetes, particularly *Streptomyces*, have historically served as a major source of antimicrobial drugs. However, discovery efforts have largely focused on environment-derived species, whereas host-associated *Streptomyces* remain underexplored despite their potential to produce specialized metabolites potentially shaped by host-microbe interactions.

Here, we investigated the metabolic and bioactive potential of 124 *Streptomyces* strains, with primary emphasis on 58 human-associated clinical isolates and 66 environmental strains used for comparison. Strains were cultivated under multiple conditions, including monoculture and co-cultivation with *Candida albicans*, to stimulate metabolite production. Haemolytic activity and *C. albicans* inhibition were recorded for integration with metabolomic profiles.

Untargeted metabolomic profiling was carried out using ultra high-performance liquid chromatography coupled with high-resolution trapped ion mobility mass spectrometry (timsTOF). Data processing was performed in MZmine 4, followed by feature-based molecular networking in GNPS2. Metabolite annotation was achieved through spectral library matching (public and in-house), complemented by in silico predictions using SIRIUS. Statistical analysis was subsequently performed to evaluate group-level differences.

The resulting molecular network comprised approximately 15,000 features, of which 15% were annotated, including siderophores, cyclo-/peptides, and known bioactive compounds such as actinomycin, bottromycin, manumycin, bafilomycin, ikarugamycin, angolamycin, and enterocin.

Univariate statistical analysis revealed a clear separation between environmental and human-associated isolates. Features upregulated in human-associated *Streptomyces* included sphingosines, phosphocholines, myristoleic acid, and ethoxylated lipids, whereas defensamide and diproton were more abundant in environmental strains. Putative mono-/di- and trinactins, as well as nalidixic acid, were detected across multiple human-associated strains but were absent from environmental isolates.

A substantial fraction of features remained unannotated in bioactive strains, while known antifungal compounds were not detected, suggesting the presence of previously uncharacterized antifungal metabolites. Integration of metabolomic, phenotypic, and genome mining data is ongoing to prioritize bioactive features and link them to biosynthetic gene clusters. Together, these results highlight human-associated *Streptomyces* as an underexplored source of specialized metabolites with potential for novel antifungal discovery.

Biogeographical landscape of microbiome derived lipids in the human colon and small intestine

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Lipids are central to cellular signaling, structure, and function across all domains of life, yet the vast majority of microbe-derived lipids in the human gut remain uncharacterized. While bile acids and short-chain fatty acids have received considerable attention, emerging evidence suggests that hundreds of thousands of microbial lipid species await discovery. In particular, the biogeographical distribution of these metabolites along the colonic axis is poorly understood, limiting our ability to link spatially organized microbial lipid metabolism to intestinal physiology and disease.

Here, we systematically profiled the lipidome across a four-vessel colon simulator (Enteromix) that recapitulates the physicochemical gradients including pH, simulation time, and substrate availability (fiber) of the human proximal-to-distal colon. Using both untargeted and targeted lipidomics, we reveal dynamic, compartment-specific shifts in a diverse repertoire of microbially derived lipids within the simulated intestinal chyme. These include novel microbial bile acids undergoing progressive biotransformation and, notably, a structurally diverse family of N-acyl amides bearing short- and odd-chain acyl groups. Histamine-linked N-acyl lipids progressively increased from proximal to distal vessels (pH 5.5–7.0), suggesting pH- or microbiota-driven biosynthesis in the distal colon. Conversely, putrescine-linked N-acyl lipids, initially abundant in the culture media, were systematically depleted along the colonic gradient, indicating active microbial consumption or biotransformation. Furthermore, supplementation with polydextrose (PDX), a prebiotic fiber, significantly modulated the abundance and distribution of these lipid species, revealing substrate-dependent regulation of the microbial lipidome. We also uncovered previously unrecognized associations between in vitro-derived short-chain N-acyl lipids and major host-relevant lipid classes, including cholesterol esters, phosphatidylethanolamines, ceramides, and sphingomyelins.

To assess the functional relevance of these findings, we employed a reverse metabolomics strategy and interrogated lipid profiles from paired human small intestinal and fecal samples using publicly available datasets, confirming the presence of key microbial lipid signatures in vivo. We further leveraged gut-derived organoids to evaluate the direct impact of candidate microbial metabolites on host epithelial responses, bridging in vitro discovery with functional exploration in a physiologically relevant cell-model system.

Together, our work establishes an integrated framework combining in vitro colonic simulation, reverse metabolomics, public repository mining, and gut cell-based functional assays for the systematic discovery and characterization of bioactive microbial lipids. These findings substantially expand the unknown chemical repertoire of the gut microbiome, highlight the biogeographical complexity of microbial lipid metabolism, and provide a roadmap for elucidating the spatial organization of lipid-microbe interactions in the human colon.

O7-1

Spatial metabolic profiling of Patient-Derived Prostate Cancer adherent primary cell cultures by MALDI Mass Spectrometry Imaging

Juan Carlos Cabellos Guillem, Victoire Bondeville, Ingrid Bjørnes Sæther, May-Britt Tessem

Patient-derived tumor models, including adherent cultures and three-dimensional organoids, represent a promising platform for studying primary prostate cancer (PCa) progression ex vivo. However, their widespread adoption remains limited by both the technical difficulty of establishing primary PCa cultures [1] and uncertainty regarding how faithfully these models preserve the molecular features of the parental tumor tissue [1]. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) offers a label-free, spatially resolved approach for assessing metabolic fidelity in these systems by enabling simultaneous profiling of lipids and small polar metabolites directly from culture preparations.

Here we present preliminary results from our MALDI-MSI protocol to study patient-derived prostate cancer adherent cells coexisting within our organoid cultures. Adherent cells obtained from cultured biopsies (N=4 patients) were grown on poly-D-lysine indium tin oxide (ITO)-coated glass slides and analyzed by MALDI-MSI using N-(1-naphthyl)ethylenediamine dihydrochloride (NEDC) matrix over an m/z range of 100–1,000.

Following acquisition, the samples were stained with hematoxylin and eosin (H&E) and co-registered for morphological validation. We identified distinct metabolite profiles across the culture areas, including the accumulation of specific phosphatidylinositol species (PI 16:0/18:1, PI 18:0/18:1, PI 18:0/20:2) linked to lipid metabolic reprogramming and the depletion of citrate and aspartate. These distributions co-localized with clusters of tightly packed cells found upon H&E co-registration, which immunofluorescence confirmed as primarily cancer epithelial cells. Overall, our adherent cultures reproduce the key metabolic hallmarks of primary PCa tissue, including lipid remodeling [2] and depletion of citrate and aspartate [3], with epithelial cells growing in clusters as previously described. These findings support MALDI-MSI as a promising platform for metabolic characterization of organoids and adherent cell cultures. This workflow is currently being extended to three-dimensional organoid systems through optimization of compatible tissue-embedding and cryosectioning protocols.

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O7-2

Spatial Glycomic and Peptidomic Phenotypes Define Primary and Recurrent Medulloblastoma

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Relapse of medulloblastoma is associated with poor prognosis and remains a major clinical challenge despite advances in frontline therapy. While genomic and transcriptomic studies have established conserved molecular subgroups and therapy-driven evolution, how tumor-associated phenotypes are spatially organized within brain tissue during primary disease and relapse remains poorly understood. Here, we applied multimodal matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) to characterize spatial glycomic and peptidomic remodeling in MYCN-driven medulloblastoma during primary tumor growth and recurrence.

Using genetically engineered mouse models that enable controlled induction of dormancy and relapse, we mapped region-resolved N-glycan and tryptic peptide distributions across sagittal brain sections while preserving tissue architecture. Comparative analysis revealed shared and tumor-enriched glycan and peptide features across medulloblastoma and surrounding cerebellar regions, consistent with the cerebellar lineage origin of the disease. Tumor-associated phenotypes emerged through altered relative abundance and spatial redistribution of molecular species.

Differential abundance analysis identified subsets of glycans and peptides preferentially enriched in tumor regions compared to matched cerebellum, including high-fucose glycans and peptides assigned to the TBB family, SYN1, MAP1B, and ROA1, with distinct spatial patterns observed at tumor cores and margins.

To assess whether these tumor-associated molecular features differed between disease stages, we compared primary tumors and recurrent lesions. This analysis revealed relapse-associated changes in the abundance and spatial organization of a subset of tumor-enriched glycan and peptide features, indicating further molecular reorganization during recurrence. These findings indicate that relapse is associated with structured, region-specific phenotypic remodeling rather than uniform tumor-wide changes. Together, these findings demonstrate that medulloblastoma is characterized by spatially organized glycomic and peptidomic phenotypes that distinguish tumor from normal cerebellum and evolve further during relapse. Multimodal MALDI-MSI provides a powerful framework to resolve tissue-level molecular heterogeneity and to interrogate phenotypic adaptations associated with brain tumor progression and recurrence.

07-3

All the sterol signals are just cholesterol: Another study on In-source Fragmentation with MALDI-2 MSI

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Elevated cholesterol levels in prostate cancer (PCa) tissue are well documented, yet we previously showed that cholesterol synthesis genes are systematically downregulated in PCa compared with normal tissue after correction for stromal content (1). This apparent contradiction suggests that PCa may shift from cholesterol synthesis toward increased import. Our original aim was to investigate this hypothesis by spatially detecting both cholesterol and synthesis pathway intermediates in PCa tissue.

Prostate tissue sections (n=34) containing both benign and cancerous morphology were coated with dihydroxybenzoic acid (DHB) and analyzed by MALDI-2 Q-TOF MSI in positive ion mode. MALDI-2 applies post-ionization which improves the detection of sterols that are otherwise difficult to ionize, but may also increase in-source fragmentation (ISF) which complicates identification by producing spectra with unintended fragment ions.

Cholesterol was readily detected at m/z 369.35 $[M-H_2O+H]^+$ and showed signal in epithelial cells, with higher intensities in cancerous compared to benign epithelium. Several additional ions, including m/z 367.34, 368.34, 385.35, and 505.37, were initially considered possible sterol intermediates, oxysterols, or related metabolites. MS/MS fragmentation indicated sterol-like structures; however, confident identification proved challenging. After investigating the literature, it became clear that under high-ionization conditions, cholesterol goes through ISF and can generate many different radicals and ion adducts with the same masses as other biologically relevant sterols (2,3). All candidate ions showed spatial distributions highly similar to cholesterol. The intensity patterns of m/z 367.33, 368.34 and 369.35 were consistent with previous MALDI-2 MSI observations reporting ISF of cholesterol (2). Based on MS/MS fragmentation, spatial distribution, and literature comparison, m/z 368.34 is likely a cholesterol-derived radical ion $[M-H_2O]^+\bullet$, m/z 385.35 likely represents cholesterol $[M+H-H_2]^+$ rather than an oxysterol, and m/z 505.37 is likely a cholesterol adduct involving a DHB fragment.

Thus, although MALDI-2 MSI enabled robust spatial detection of cholesterol in prostate tissue, the study also revealed a substantial risk of misidentifying cholesterol-derived ions as other sterols. These findings emphasize the need for careful annotation strategies in sterol MSI and are important for avoiding misleading biological conclusions.

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Lightning Talks

5th Nordic Metabolomics Conference 2026

Linking Individual Urine Metabolomic Profiles to Postprandial Responses in the NUTRIOME Study

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Diet is a key factor in preventing non-communicable diseases, but individuals respond differently to the same foods, highlighting the need for personalised nutrition. The NUTRIOME meal study, a data-driven precision nutrition intervention study using meal responses, is a two-phase, multi-centre precision nutrition intervention, aimed at developing tailored dietary recommendations to reduce cardiometabolic risk factors and investigating factors driving individual differences in postprandial responses. Urine is used as a non-invasive biological sample to capture host-, microbial-, and diet-derived metabolites, enabling detailed analysis of individual metabolic profiles. The aim of this project is to investigate whether baseline urinary metabolomic profiles differ across distinct postprandial response types and to examine their associations with metabolic and immune responses.

Ninety-seven overweight adult participants (74 females, 23 males) consumed, in a crossover design, three isocaloric meals differing in macronutrient composition (carbohydrate-rich, high-quality fat-rich, and low-quality fat-rich), once a week over three consecutive weeks, while consuming their habitual diet on all other days. Blood samples were collected before and after meal consumption to assess postprandial immune and metabolic responses, including glucose, triglycerides and interleukin-6. On each study day, fasting first-morning urine samples were collected before consumption of the test meal. The three urine samples of each participant were subsequently analysed using high-performance liquid chromatography–mass spectrometry (HPLC-MS)-based untargeted metabolomics.

Using the postprandial responses to the three meal tests, an algorithm was developed to stratify individuals into three response types. A total of 3,094 compounds were detected across all samples (MSI level 1: n=212; level 2: n=527; level 3: n=1918; level 4: n=1247), providing broad coverage of the semi-polar urine metabolome. Inter-individual differences were observed, indicating the highly personalized nature of urine metabolomic profiles and highlighting the importance of considering individual variability in human metabolomics studies. Ongoing analyses are exploring differences in urine metabolites between response types, as well as associations between the urine metabolome and postprandial metabolic responses. These findings may provide further insight into how individual urine metabolomic profiles, including microbial-derived metabolites, relate to metabolic and immune responses following food intake.

Elucidating the metabolic characteristics in the developing human liver through genome-scale metabolic modelling

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Per- and polyfluoroalkyl substances (PFAS) are endocrine disrupting chemicals that continue to be used in many industrial products. PFAS exposure alters metabolite levels including bile acids, increasing susceptibility to the onset and severity of various non-communicable diseases. Biomonitoring studies reveal that PFAS are detected in almost all humans, including fetuses, raising serious concerns for long-term health. Recently, we reported that PFAS accumulate in the fetal human liver and are associated with disturbances in lipid metabolism, including bile acid metabolism (1). Therefore, identification of specific metabolic pathway perturbations caused by PFAS exposure in the human fetus is needed. Here, we investigated metabolic pathways of the human fetal liver using omics data and a constraint-based modelling approach. We integrated liver proteomics data from 56 human fetuses (gestational ages between 12 and 19 weeks) with a comprehensive genome-scale metabolic model, Human1 (2), to predict metabolic flux profiles. Our analysis revealed metabolic reprogramming in the developing liver, as well as its dysregulation by PFAS exposure. We observed that replication-related pathways are more active at early gestational age (12–14 weeks). Furthermore, we identified several PFAS-associated metabolic signatures, specifically highlighting alterations in lipid, amino acid, nucleotide, pyruvate, glycan and vitamin metabolism. Interestingly, model predictions revealed that PFAS negatively impact steroid metabolism, including estrogen and androgen metabolism. To confirm the impact of PFAS exposure on the fetal liver steroidome, we analysed liver samples from an independent cohort of 84 fetuses (gestational ages between 11 and 20 weeks) by both targeted (steroids) and untargeted mass spectrometry-based metabolomics analyses. Androstenedione was inversely ($\beta = -0.34$, $p < 0.001$) and 17β -estradiol ($\beta = 0.21$, $p = 0.042$) was positively associated with PFAS. Moreover, ratios of substrate steroids over product steroids were inversely associated with PFAS, indicating that PFAS disrupt steroidogenic enzyme activity. In conclusion, PFAS exposure is associated with disturbed liver development, including altered production of important steroids.

The abundance and therapeutic potential of lipid droplets in Multiple Myeloma

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Multiple myeloma (MM) is a heterogeneous haematological malignancy originating from the bone marrow. One of the main challenges facing patients diagnosed with MM, is that despite the recent advances in treatment and disease management, many patients will eventually relapse and develop treatment resistance. Interestingly, in MM, no single genetic alteration has been established as principal pathogenic event highlighting the role of the intertwined dysregulation of several molecular mechanisms, including metabolic reprogramming, in MM development. Notably, malignant plasma cells dynamically alter key metabolic pathways to support their elevated energy demands resulting in disease progression. Previous studies have suggested that cancer cells meet their increased energetic demands needed to maintain a high proliferative rate by enhancing lipid synthesis and storage within LDs. Moreover, elevated LD abundance has been linked to treatment resistance. However, the dependency of LDs in MM has not been investigated yet.

This research aims to evaluate the effect of de novo lipogenesis inhibition on LDs in human and murine MM cells. We identified increased abundance of LDs in MM cells by LD-BTD1 staining. By utilizing RNA-seq data from newly diagnosed MM patients within the MMRF cohort, we demonstrated that key gene transcripts of the de novo lipogenesis pathway (ACLY and SREBP1) were overexpressed in MM compared to healthy individuals and associated with worse prognosis. Interestingly, inhibition of ACLY (BMS-303141) and SREBPs (fatostatin) significantly reduced cell viability and induced apoptosis in human and murine MM cells. Furthermore, combinatorial treatment with bortezomib, the standard-of-care for newly diagnosed MM patients, and BMS-303141/fatostatin significantly increased cell death and apoptosis in human and murine MM cells compared to control and the single treatments. To shed light on the underlying mechanisms promoting these anti-MM effects, we will investigate the downstream impact of the treatments via metabolic and lipidomic profiling, using liquid chromatography coupled with mass spectrometry and by RNA-seq. To investigate the therapeutic potential of the combinatorial treatment in vivo, we will evaluate its effect on tumour growth and mouse survival in the 5T33MM immunocompetent syngeneic murine MM model and in an OPM2 xenograft (CDX) MM model.

Taken together, our preliminary data suggest that MM cells rely on lipid droplets for their survival and proliferation. Moreover, we demonstrated that combination of bortezomib with de novo lipogenesis inhibition enhanced the sensitivity of human and murine MM cells to the standard-of-care suggesting a novel therapeutic avenue for the treatment of MM.

Exercise as a Modulator of the Human Milk Lipidome

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Human milk is a compositionally dynamic biofluid whose lipid fraction provides approximately 50% of infant caloric intake and plays critical roles in neonatal neurodevelopment, immune maturation, and long-term metabolic programming. Beyond energy provision, the human milk lipidome comprises structurally diverse lipid classes, including triacylglycerols, phospholipids, sphingolipids, and bioactive lipid mediators with important signaling and metabolic functions. Advances in lipidomic technologies, particularly liquid chromatography-ion mobility-mass spectrometry (LC-IMS-MS), have expanded characterization of the human milk lipidome, revealing considerable inter-individual variability influenced by lactation stage, maternal body composition, diet, and metabolic status. Maternal obesity has been associated with a shift toward saturated fatty acid species at the expense of functional lipids. Emerging evidence further indicates that maternal exercise during lactation may modulate milk lipid composition by remodeling mammary metabolism and increasing the concentration of lipid species linked to reduced adiposity, such as 12,13-diHOME. However, the influence of exercise intensity on the human milk lipidome remains largely uncharacterized.

This randomized crossover study investigated the acute effects of maternal exercise intensity on the human milk lipidome in lactating individuals (n=27) with overweight or obesity (BMI ≥ 25 kg/m²) at 6-8 weeks postpartum. Participants completed three conditions in randomized order: rest, moderate-intensity continuous training (MICT), and high-intensity interval training (HIIT). Milk samples were collected at multiple time points (T0-T4) per condition and analyzed using a targeted lipidomic platform (Complex Lipid Targeted Panel, Metabolon Inc.) capable of quantifying up to 1,100 lipid species across major lipid classes. Following stringent quality control, the effects of condition and time were assessed using linear mixed models and repeated-measures ANOVA simultaneous component analysis (RM-ASCA).

Among 828 lipid species analyzed, 805 showed significant temporal variation, of which 791 remained significant after multiple-testing correction. For time-condition interactions, 12 species differed between MICT and rest, with none surviving correction. In contrast, HIIT versus rest yielded 130 significant species, with one (DAG18:2/20:5) remaining significant after multiple-testing correction. Among 14 lipid classes examined, 12 displayed similar time-condition interaction patterns. Diacylglycerol (DAG) and dihydroceramide (DCER) were notable exceptions, showing persistently elevated levels during rest compared with both exercise conditions across time points. In HIIT condition, triacylglycerol species enriched in saturated fatty acid were elevated, consistent with the overall elevation in total fatty acid content. These findings indicate that acute maternal exercise selectively modulates the human milk lipidome in an intensity-dependent manner.

Persistent reprogramming of host lipid metabolism following *Rickettsia akari* infection

Petra Chalova, Marco Quevedo Diaz, Ludovit Skultety, Andrej Kovac, Alex Dickens

Rickettsia akari, the causative agent of rickettsialpox, manipulates host cell metabolism during acute infection, yet whether these metabolic perturbations persist long-term remains unknown. Using high-sensitivity liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), we characterized the plasma lipidome across two complementary study designs to investigate population and individual level metabolic shifts. A cross-sectional analysis of 40 confirmed rickettsiosis patients and 15 healthy controls established clear lipidome based group separation, with triacylglycerols (TG), phosphatidylcholines (PC), and cholesterol esters (CE) as the primary discriminating lipid classes. Second, a longitudinal case study tracked a single *R. akari* infected patient across 11 years: pre-infection (2014), acute phase (2017), and eight years post-infection (2025). While the acute phase showed a broad, transient suppression of circulating lipids, the 2025 lipidome did not return to baseline. Instead, we observed sustained remodeling characterized by persistently elevated CE(20:5) and CE(22:6), alongside chronic depletion of diacylglycerols (DG) particularly DG(42:6) and free fatty acids (FA). This inverse relationship between esterified storage lipids and bioactive signaling lipids suggests a permanent shift toward lipid storage overactive utilization, potentially indicative of long-term mitochondrial dysfunction. These results highlight a novel phenomenon of pathogen-induced metabolic imprinting, where a transient infection triggers a permanent remodeling of the host plasma lipidome. Our findings demonstrate that rickettsial infection can induce metabolic reprogramming that outlasts clinical resolution by years, providing new insights into the post-infectious chronic phase and opening new approaches for basis of differential metabolic profiling of *Rickettsia* species.

Beyond Glucose Transport: Off-Target Effects of SGLT2 Inhibitors Reveal Mitochondrial and Transporter Remodeling in Kidney Disease

Anja M. Billing, Young Chul Kim, Elina Kovalenko, Vera C. Wulfmeyer, Volker Vallon, Markus M. Rinschen

Introduction: Sodium–glucose cotransporter 2 inhibitors (SGLT2i), originally developed to lower hyperglycemia, confer robust renal and cardiovascular protection even in non-diabetic individuals. These benefits cannot be fully explained by inhibition of renal glucose reabsorption, suggesting additional, SGLT2-independent mechanisms.

Methods: To identify off-target effects, we performed integrated proteomic and metabolomic profiling using LC–MS/MS in SGLT2 knockout (SGLT2KO) and wild-type mice across four conditions: healthy, diabetes, obesity, and nephropathy. Disease models included Western diet-induced obesity, Akita-driven type 1 diabetes, and aristolochic acid-induced nephropathy. Cross-species validation was achieved by comparing murine data with plasma and urine profiles from individuals with familial renal glucosuria (genetic SGLT2 deficiency) and SGLT2i-treated patients with and without chronic kidney disease. Direct protein–drug interactions were assessed using thermal proteome profiling in kidney tissue, complemented by short-term pharmacological studies in a microfluidic system. Proteomics was performed label-free on an Exploris 480 platform, and targeted metabolomics on a triple-quadrupole LC–MS.

Results: We generated a comprehensive multi-model atlas of SGLT2-dependent and -independent effects. SGLT2KO signatures were conserved between mice and humans, validating translational relevance. Notably, SGLT2i induced substantial off-target remodeling of renal transport processes, with the strongest effects observed in nephropathy. Chronic kidney disease was characterized by pronounced mitochondrial dysfunction, which was partially rescued by SGLT2i independently of SGLT2. Thermal proteome profiling identified direct binding to multiple solute carrier (SLC) transporters and components of the mitochondrial respiratory chain.

Conclusions: SGLT2 inhibitors exert significant SGLT2-independent effects in the kidney, targeting SLC transporters and mitochondrial pathways. Modulation of mitochondrial function may contribute to their cardio-renal protective phenotype. These findings expand the mechanistic framework of SGLT2i and highlight novel targets for therapeutic intervention.

Pushing detection limits in clinical metabolomics: enhanced dynamic range scan mode across conventional and microsampling applications

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Global metabolomics is increasingly applied in clinical contexts, from biomarker discovery and patient stratification to monitoring of microbiome–host interactions and inborn errors of metabolism. However, its clinical translation is constrained by the analytical performance of conventional LC-MS workflows, which struggle to balance sensitivity, dynamic range, and metabolome coverage in complex biological samples. The challenge is most acute for clinically relevant low-abundance compounds, such as microbial metabolites, signaling molecules, hormones, and in microsampling applications, e.g. dried blood spots (DBS), where small volumes push relevant metabolite levels close to the limit of detection. Improving instrument-level sensitivity is therefore a prerequisite for routine, deep clinical metabolomics.

Here, we benchmarked enhanced Dynamic Range (eDR) full-scan acquisition combined with intelligent MS/MS triggering (AcquireX) on a modified Orbitrap-Hybrid-mass-spectrometer (mOH) against the routine QExactive Orbitrap (QE) workflow used in our clinical metabolomics laboratory. The platforms were compared across a hospital-derived pooled plasma reference material (~4,000 patients aged 0–94 years), pooled DBS from healthy volunteers, and patient samples from multiple matrices and pathological states. Methanol extraction and reversed-phase HPLC (Pursuit XRs Diphenyl, 47.5 min gradient) were used in positive ionization mode, with data processed in Compound Discoverer 3.5.

In plasma, eDR clearly extended metabolome and xenobiotic coverage compared with conventional full scan, with mzCloud spectral matches increasing by ~29% and corresponding gains in in-house library annotations. Signal-to-noise (S/N) for low-abundance metabolites improved markedly, for example, serotonin S/N increased from 139 (QE) to 287 (mOH full-scan) and 547 (mOH eDR), while the gut microbial–derived metabolite trimethylamine N-oxide showed a 26% S/N gain with eDR. The number of detected medications more than doubled with eDR (from 8 to 18, including warfarin, metoclopramide, diazepam), highlighting added value for clinical pharmacology and toxicology.

The strongest gains, however, were observed in microsampling. In DBS, the QE detected 310 compounds versus 941 on the mOH, with in-house library matches rising from 36 to 73 and mzCloud matches from 51 to 177. Clinically informative metabolites such as paraxanthine, phosphorylcholine, and cystine were detected exclusively on the mOH, with eDR providing further gains up to 5-fold for cystine, 2.5-fold for serine, and 62% for creatinine, a key kidney function marker. Microbially derived indole-3-acrylic acid and N,N,N-trimethyllysine showed up to 5-fold and 2.6-fold S/N improvements over the routine QE workflow.

Enhanced dynamic range Orbitrap-mass-spectrometry meaningfully extends coverage in standard clinical matrices and truly shines in microsampling, enabling deeper, more sensitive clinical metabolomics analysis.

Spatial metabolomics using capillary electrophoresis-mass spectrometry with hydrodynamic injection

Natalia Remper, Ingela Lanekoff

Capillary electrophoresis-mass spectrometry (CE-MS) is a powerful analytical technique for separating and detecting metabolites in chemically complex samples. However, samples for CE-MS are typically homogenized prior to analysis, which results in the loss of spatial information on metabolite localization. To retain the spatial information and still take advantage of CE-MS, we developed surface-sampling CEMS (SS-CE-MS), which enables direct sampling from specific tissue regions while preserving spatial information. In previous work, electrokinetic injection was used for sample transfer into the separation capillary. Here, we present a redesigned SS-CE-MS platform employing hydrodynamic injection to minimize potential sampling biases and improve sampling reproducibility for spatial metabolomics.

The SS-CE-MS platform consisted of fused silica capillaries for analyte extraction and separation, a high-voltage power supply (Spellman SL10) operating at 22 kV, and motorized linear stages (Zaber) for sample positioning during hydrodynamic injection. The system was coupled to an Orbitrap IQ-X Tribrid mass spectrometer (Thermo Fisher Scientific) for high-resolution detection. Surface sampling was achieved by forming a microdroplet between the tissue surface and the probe, allowing localized analyte extraction prior to hydrodynamic injection and electrophoretic separation.

The performance of the platform was evaluated using metabolite standards and mimetic tissue sections. The results demonstrated reproducible separation and relative migration times of metabolites. Within 25 min, a wide range of metabolites extracted directly from the tissue were separated and detected, including amino acids, neurotransmitters, and isomeric compounds such as valine and betaine. The sampling probe was redesigned to achieve a reduced and consistent sampling spot size of 278 μm with 1 % relative standard deviation, compared with previous electrokinetic SS-CE-MS designs. To increase the throughput, multisegment injections directly from different locations on the tissue surface were performed. Consecutive sampling from three distinct locations within a single CE run increased throughput nearly threefold while maintaining consistent analyte separation and migration behavior across all sampled segments.

Overall, the newly developed SS-CE-MS platform enabling hydrodynamic injection was found to provide reproducible, spatially resolved analysis of diverse metabolites with isomeric specificity and improved sampling precision, demonstrating strong potential for spatial metabolomics studies of biological tissues.

Posters

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Whole-Grain Rye feeding Drives Tissue-Specific Host–Microbial Metabolic Responses Beyond Conventional Clinical Endpoints in a mouse model

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Dietary interventions for metabolic diseases are evaluated using circulating clinical risk markers; however, they provide limited insight into tissue-specific mechanisms and may overlook functional differences between diets with similar clinical phenotypes. This limitation is particularly relevant for complex whole foods such as whole-grain rye (WGR), a Nordic staple commonly consumed as sourdough-fermented bread, where fermentation may alter nutrient and phytochemical availability, fiber structure, and microbial accessibility.

To investigate whether fermentation shapes host metabolic responses to rye bread (RB) that are not captured by conventional clinical endpoints, mice were fed low-fat (LF) diets supplemented with fermented (LF-FRB) or unfermented rye bread (LF-URB). Systemic and tissue-specific responses were assessed by integrating clinical measures, ¹⁸F-FDG PET imaging to quantify tissue glucose uptake in the brain, intestine, heart, and liver; untargeted metabolomics across plasma, heart, muscle, intestinal tissues, and feces; and fecal metagenomics.

LF-background enabled detection of functional metabolic responses to WGR without confounding diet-induced metabolic disturbances. Despite indistinguishable clinical phenotypes (body weight and plasma glucose, insulin, liver, and lipid parameters) and glucose uptake in the brain, intestine, heart, and liver across diets, both RBs showed higher betaines and tryptophan-derived microbial metabolites along the gut–plasma axis. However, fermentation produced distinct tissue metabolic signatures: LF-FRB uniquely promoted lipid remodeling in muscle and intestinal tissues, characterized by increased acylcarnitines, glycerophospholipids, sphingolipids, and monoacylglycerols, whereas LF-URB showed higher antioxidant compounds (e.g., ergothionine) and lowered potentially harmful microbial metabolites, including p-cresol sulphate. Metagenomic analysis revealed diet-associated shifts in microbial taxa, including lower *Adlercreutzia mucosicola* and higher *Lachnospiraceae* taxa in both RBs and lower *Bacteroides acidifaciens* in LF-URB; all previously linked to mucosal and metabolic functions in perturbed contexts.

These findings provide the first systems-level multi-organ characterization of WGR, revealing fermentation-driven metabolic and microbial divergence despite indistinguishable clinical phenotypes. Integrated multi-compartment metabolomics and metagenomics indicate that tissue-level metabolic responses to food-processing may precede measurable clinical changes, highlight early metabolic adaptations and provide mechanistic insight into diet–microbiome interactions relevant to metabolic health

PFAS Exposure Modulates Mitochondrial Susceptibility in Steatotic Hepatocytes

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants increasingly associated with metabolic dysfunction-associated steatotic liver disease (MASLD). As mitochondria are central regulators of hepatic energy production, lipid handling, and stress responses, mitochondrial metabolism may represent a sensitive target of PFAS toxicity, particularly under steatotic conditions. However, most PFAS studies have relied on whole-cell or biofluid analyses, leaving organelle-enriched metabolic responses insufficiently characterized. Here, we investigated how a human-relevant PFAS mixture modulates mitochondrial metabolism in non-steatotic and MASLD-like steatotic human HepaRG hepatocytes. HepaRG cells were exposed for 24 h to a mixture of 47.8% PFOS, 28.7% PFOA, 12.1% PFHxS, 6.3% PFDA, and 5.1% PFNA, with a total PFAS concentration of $22.44 \mu\text{mol L}^{-1}$, reflecting proportions commonly reported in human biomonitoring studies. PFAS exposure was assessed alone and in combination with palmitate/oleate-induced steatosis. Mitochondria-enriched fractions and extracellular media (secretome) were analyzed using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS), covering polar and semipolar metabolites, tricarboxylic acid cycle intermediates, and molecular lipids. These molecular profiles were integrated with Seahorse-based mitochondrial respiration analysis. Steatosis induced marked lipid remodeling in mitochondria-enriched fractions, including accumulation of triacylglycerols and depletion of phosphatidylcholines, phosphatidylethanolamines, sphingomyelins, and cholesteryl esters, consistent with altered mitochondrial–lipid droplet interactions. PFAS exposure alone produced a distinct mitochondrial metabolic fingerprint, including cardiolipin-associated alterations and associations between individual PFAS and key metabolites linked to mitochondrial energy metabolism. Importantly, the combined PFAS and steatosis condition produced the strongest metabolic perturbation, with broad depletion of membrane lipid classes, cardiolipin-associated disruption, reduced carnitine levels, increased triacylglycerols, and elevated extracellular acylcarnitines, indicating impaired fatty-acid handling and mitochondrial inner membrane stress. Pathway analysis highlighted disturbances in lipid and inflammatory metabolism, including leukotriene metabolism and squalene/cholesterol biosynthesis, as shared nodes across PFAS exposure and steatosis. Functional assays further showed that steatosis increased basal respiratory activity while nearly abolishing spare respiratory capacity, whereas PFAS exposure suppressed respiratory capacity and reduced the energetic flexibility of steatotic hepatocytes. Together, these findings demonstrate that steatotic hepatocytes are more susceptible to PFAS-induced mitochondrial metabolic disruption than non-steatotic cells. This study identifies mitochondrial lipid remodeling, cardiolipin-associated alterations, and acylcarnitine release as candidate signatures of PFAS-related mitochondrial vulnerability in fatty liver disease.

Development and Validation of a UHPLC-MS/MS Method for Simultaneous Quantification of Dietary Sugars and Non-Nutritive Sweeteners in Human Urine

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Background: Excessive consumption of dietary sugars and increasing use of non-nutritive sweeteners (NNS) are associated with metabolic disorders including obesity, type 2 diabetes, non-alcoholic fatty liver disease, and cardiovascular disease. Objective assessment of these dietary exposures is challenging because conventional dietary questionnaires are prone to reporting bias. Urinary sugars and NNS offer reliable biomarkers of recent intake; however, their simultaneous quantification is analytically challenging due to structural diversity, low concentrations, and matrix complexity. This study aimed to develop and validate a robust, high-throughput UHPLC-MS/MS method for the simultaneous determination of dietary sugars and NNS in human urine.

Methods: Urinary sugars were derivatized using 3-nitrophenylhydrazine (3-NPH) and analyzed by UHPLC-MS/MS using a pentafluorophenyl (PFP) stationary phase. The method was optimized to achieve efficient chromatographic separation of critical sugar isomers while enabling simultaneous quantification of urinary NNS. Method validation included evaluation of sensitivity, linearity, accuracy, precision, recovery, matrix effects, and analyte stability. Robustness and applicability were assessed through analysis of 2,500 spot urine samples from participants in the Swedish CARDioPulmonary bioImage Study (SCAPIS).

Results: The derivatization-based PFP approach provided excellent chromatographic performance, resolving key sugar isomers and overcoming limitations encountered with HILIC-based methods, including retention instability and poor peak reproducibility. The assay demonstrated high sensitivity, broad linear ranges, and satisfactory accuracy and precision across all analytes. Recovery, matrix effects, and stability assessments supported reliable quantification in the complex urinary matrix. Application to 2,500 human urine samples confirmed method robustness, reproducibility, and suitability for large-scale population studies.

Conclusions: A sensitive, selective, and high-throughput UHPLC-MS/MS method was successfully developed and validated for simultaneous measurement of urinary sugars and non-nutritive sweeteners. The method enables objective assessment of dietary exposure to both caloric and non-caloric sweeteners and is well suited for large epidemiological and nutritional intervention studies. Its successful deployment in 2,500 urine samples demonstrates its potential as a powerful tool for dietary biomarker research and investigation of sugar replacement patterns in human populations.

Persistent reprogramming of host lipid metabolism following *Rickettsia akari* infection

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Rickettsia akari, the causative agent of rickettsialpox, manipulates host cell metabolism during acute infection, yet whether these metabolic perturbations persist long-term remains unknown. Using high-sensitivity liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), we characterized the plasma lipidome across two complementary study designs to investigate population and individual level metabolic shifts. A cross-sectional analysis of 40 confirmed rickettsiosis patients and 15 healthy controls established clear lipidome based group separation, with triacylglycerols (TG), phosphatidylcholines (PC), and cholesterol esters (CE) as the primary discriminating lipid classes. Second, a longitudinal case study tracked a single *R. akari* infected patient across 11 years: pre-infection (2014), acute phase (2017), and eight years post-infection (2025). While the acute phase showed a broad, transient suppression of circulating lipids, the 2025 lipidome did not return to baseline. Instead, we observed sustained remodeling characterized by persistently elevated CE(20:5) and CE(22:6), alongside chronic depletion of diacylglycerols (DG) particularly DG(42:6) and free fatty acids (FA). This inverse relationship between esterified storage lipids and bioactive signaling lipids suggests a permanent shift toward lipid storage overactive utilization, potentially indicative of long-term mitochondrial dysfunction. These results highlight a novel phenomenon of pathogen-induced metabolic imprinting, where a transient infection triggers a permanent remodeling of the host plasma lipidome. Our findings demonstrate that rickettsial infection can induce metabolic reprogramming that outlasts clinical resolution by years, providing new insights into the post-infectious chronic phase and opening new approaches for basis of differential metabolic profiling of *Rickettsia* species.

Linking Individual Urine Metabolomic Profiles to Postprandial Responses in the NUTRIOME Study

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Diet is a key factor in preventing non-communicable diseases, but individuals respond differently to the same foods, highlighting the need for personalised nutrition. The NUTRIOME meal study, a data-driven precision nutrition intervention study using meal responses, is a two-phase, multi-centre precision nutrition intervention, aimed at developing tailored dietary recommendations to reduce cardiometabolic risk factors and investigating factors driving individual differences in postprandial responses. Urine is used as a non-invasive biological sample to capture host-, microbial-, and diet-derived metabolites, enabling detailed analysis of individual metabolic profiles. The aim of this project is to investigate whether baseline urinary metabolomic profiles differ across distinct postprandial response types and to examine their associations with metabolic and immune responses.

Ninety-seven overweight adult participants (74 females, 23 males) consumed, in a crossover design, three isocaloric meals differing in macronutrient composition (carbohydrate-rich, high-quality fat-rich, and low-quality fat-rich), once a week over three consecutive weeks, while consuming their habitual diet on all other days. Blood samples were collected before and after meal consumption to assess postprandial immune and metabolic responses, including glucose, triglycerides and interleukin-6. On each study day, fasting first-morning urine samples were collected before consumption of the test meal. The three urine samples of each participant were subsequently analysed using high-performance liquid chromatography–mass spectrometry (HPLC-MS)-based untargeted metabolomics.

Using the postprandial responses to the three meal tests, an algorithm was developed to stratify individuals into three response types. A total of 3,094 compounds were detected across all samples (MSI level 1: n=212; level 2: n=527; level 3: n=1918; level 4: n=1247), providing broad coverage of the semi-polar urine metabolome. Inter-individual differences were observed, indicating the highly personalized nature of urine metabolomic profiles and highlighting the importance of considering individual variability in human metabolomics studies. Ongoing analyses are exploring differences in urine metabolites between response types, as well as associations between the urine metabolome and postprandial metabolic responses. These findings may provide further insight into how individual urine metabolomic profiles, including microbial-derived metabolites, relate to metabolic and immune responses following food intake.

P6

Comparative Analysis of Bruker and Nightingale NMR Platforms for Metabolite and Lipoprotein Profiling

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Nuclear magnetic resonance (NMR) spectroscopy is ideal for large-scale metabolomics studies. Two leading platforms for automatic metabolite and lipoprotein subfraction quantification from NMR spectra are available through Bruker BioSpin and Nightingale Health. These platforms however differ in pulse sequences, quantification algorithms, and metabolite coverage, hampering the translation of findings across studies.

We here present a comprehensive comparison of metabolite and lipoprotein measurements obtained from 200 matched blood samples from healthy participants of the Trøndelag Health (HUNT) Study analyzed using the Bruker IVDr and Nightingale Health NMR platforms. We evaluate measurement agreement and explore the potential for cross-platform translation of measured blood markers. Both platforms showed high correlations with clinical measurements of serum lipids, creatinine and glucose. Lipoprotein main fractions demonstrated high cross-platform consistency, except for IDLs, whereas subfractions revealed notable differences in correlation structures within each platform. In specific, a higher specificity for certain lipoprotein features was found for the Bruker B.I.LISA method. For small-molecule metabolites, platform-specific correlations were generally lower and varied in strength. Therefore, comparisons of research findings across these two NMR platforms should be interpreted with caution in specific for lipoprotein subfractions and specific metabolites.

Associations between circulating metabolites and incidence of breast, prostate, lung and colorectal cancers in the HUNT Study and UK Biobank

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Background: Breast, prostate, lung and colorectal cancers are the most common cancers in both women and men globally. The role of circulating metabolites, including lipoprotein subfractions, in the development of these cancers remains unclear. This prospective cohort study aimed to explore the associations between circulating metabolites and the incidence of these cancers.

Methods: From two large population-based cohorts, the Trøndelag Health Study (HUNT) and the UK Biobank (UKBB), 132 lipoprotein subfractions and metabolites, quantified using nuclear magnetic resonance spectroscopy, were available for 497,717 participants. Cox regression was used to investigate the associations with incident cancers.

Results: During a mean follow-up time of 14 years, 549 women and 741 men in HUNT3 and 12,112 women and 14,749 men in UKBB developed breast, prostate, lung or colorectal cancer. In the UKBB, higher levels of several subfractions of lipoproteins including VLDL and LDL were associated with a decreased incidence of prostate and lung cancers (cholesterol in large LDL: HR per 1-SD increment 0.84, 95% CI 0.81-0.87 for lung cancer). Similar associations were observed between albumin, some fatty acids, and lung cancer. However, no clear associations were observed in HUNT3.

Conclusion: Several lipoprotein subfractions and circulating metabolites were associated with a reduced incidence of lung cancer, predominantly in UKBB. Although no specific lipid or metabolite profile was associated with the most common cancers, the observed associations were generally consistent across cohorts. Our results highlight promising directions for future research into metabolic biomarkers and their potential role in cancer prevention.

Bridging global metabolomics from research to clinical practice: a hospital-derived plasma reference material for sustainable long-term quality control

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Global metabolomics has clear potential to transform clinical practice, from biomarker discovery and diagnosis of inborn errors of metabolism to therapeutic monitoring and personalized medicine. Yet its translation from research into routine clinical laboratories remains slow. One of the major bottlenecks is the lack of sustainable, clinically representative reference materials for long-term quality control (LTQC), method validation, and harmonization across laboratories and over time.

The metabolomics community has primarily relied on commercially available reference materials, such as NIST SRM 1950 plasma (NIST-plasma). While valuable for the field, this material has critical limitations for clinical use: donors represent only healthy individuals within a narrow age range (40-50 years), the metabolic and xenobiotic complexity of patient samples is poorly captured, and the material is expected to expire in 2028. Since many clinically relevant metabolites become abundant only in specific pathological conditions or following pharmacological intervention, clinical metabolomics laboratories cannot rely on this resource alone.

To address this gap, we developed a hospital-derived pooled plasma reference material, Oslo-LTQC, at Oslo University Hospital, one of Europe's largest hospitals. Oslo-LTQC reflects the biochemical complexity of nearly 4,000 individuals (aged 0-94 years), encompassing virtually all diagnoses and treatments encountered in clinical routine. Preparation followed ISO/TR 33402:2025, and characterization was performed according to ISO 33405:2024 using certified clinical laboratory analyses combined with global metabolomics (PMID:34296888) and lipidomics (PMID:40825638). Several batch-correction strategies were evaluated to support long-term and multi-batch studies.

Compared to NIST-plasma, Oslo-LTQC yielded 36% and 15% more detected compounds in positive and negative ionization mode, respectively. Numerous endogenous and exogenous compounds were detected exclusively in Oslo-LTQC, including 4-hydroxyphenylacetate, N-acetyltryptophan, and a broad range of xenobiotics (e.g. warfarin, metoclopramide, diazepam). Several clinically relevant endogenous metabolites also showed significantly higher levels (e.g. bile acids: $\log_2FC = 1.6$, $p = 1.3 \times 10^{-10}$ for glycochenodeoxycholate). Importantly, global metabolomics and lipidomics profiles of Oslo-LTQC more closely resembled those of authentic clinical samples (clustering on a PCA score plot) than NIST-plasma did, supporting its suitability for clinical workflows.

Implementing global metabolomics in clinical laboratories requires reference materials that reflect true patient diversity and remain reliably available over time. Oslo-LTQC provides a sustainable, metabolically rich, and clinically representative resource that directly addresses one of the central bottlenecks for clinical translation of global metabolomics across international laboratories.

The effect of lifestyle, body composition, and medications on steroid profile in men undergoing prostate cancer diagnostics

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Endogenous steroid hormones regulate numerous physiological processes in men. Lifestyle factors may significantly influence steroid profiles. However, understanding of how these factors influence steroid levels remains limited. The goal of the current research was to examine how lifestyle, including diet and physical activity, body composition, and medication influence the steroids profiles.

This study was carried out as part of the PROMIC cohort (Prospective Translational Study on Microbiota and Prostate Cancer), conducted University of Turku in collaboration with Turku University Hospital. The study included 325 men with prostate cancer suspicion. All participants underwent a detailed medical history, lifestyle and dietary questionnaires, weekly physical activity monitoring, and abdominal MRI with consecutive body composition assessment. The body composition assessment included subcutaneous adipose tissue, visceral adipose tissue, liver fat content, and muscle mass evaluation. Subsequently, a profile of 21 steroids, comprised androgens, keto-androgens, progestogens, mineralocorticoids, glucocorticoids, estrogens was determined using HPLC-MS/MS. Statistical analysis revealed that visceral adipose tissue was the most significant contributor to differences in steroid profiles in the study cohort. The next most significant factors were waist circumference, body mass index, smoking status, and age. Androgens showed a consistent negative correlation with adipose tissue-related variables and a positive correlation with physical activity parameters. Progestogens showed a negative correlation with adipose tissue. Significant changes in the levels of eight steroids were observed in patients receiving 5 α -reductase inhibitors (5-ARI; N=49). The most pronounced effects were for androgens directly involved in the 5 α -reductase pathway: dihydrotestosterone was lower in the 5-ARI group (median 409 vs 1294 pM; FC 0.32; $p < 0.001$), while testosterone was significantly higher (median 17827 vs 13988 pM; FC 1.27; $p < 0.001$). Androsterone (median 117 vs 330 pM; FC 0.35; $p < 0.001$) and 17 α -hydroxypregnenolone were downregulated by 5-ARI usage (median 6482 vs. 7700 pM; FC 0.84; $p = 0.029$). Corticosterone (median 7352 vs. 6139 pM; FC 1.20; $p = 0.022$), 11 β -hydroxyandrostenedione (median 8250 vs. 6350 pM; FC 1.30; $p = 0.035$), androstenedione (median 2775 vs 2094 pM; FC 1.33; $p < 0.001$), and estrone concentrations were elevated in patients receiving 5-ARI (median 220 vs 160 pM; FC 1.38; $p < 0.001$).

Steroid hormone profiles in men are strongly influenced by lifestyle and body composition factors, with visceral adipose tissue as the most important determinant. Additionally, 5 α -reductase inhibitor therapy significantly alters the steroid pathway, highlighting the importance to consider medication while interpreting steroid profiles in clinical and research

Microsampling for Longitudinal Exposome and Postprandial Metabolomics Analysis

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Comprehensive exposome and metabolome profiling requires sampling strategies that enable frequent, minimally invasive collection while maintaining analytical robustness. In this study, we evaluated quantitative blood microsampling as a platform for longitudinal exposome and metabolomics investigations by combining repeated real-world sampling with controlled dietary challenge testing.

First, we assessed the stability and reproducibility of microsampling using reference blood and optimised the extraction method for the collected whole blood pellets, using a 96-well plate system. With healthy volunteers, we applied the microsampling during a three-week longitudinal study involving daily sample collection. Concurrently, lifestyle-related data, including sleep, physical activity, dietary habits, and menstrual cycle patterns, were collected throughout the study period. Samples were analyzed using three complementary ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) platforms, enabling broad molecular coverage and high analytical sensitivity. The analytical workflow allowed reliable detection of both endogenous metabolites and exogenous compounds, including per- and polyfluoroalkyl substances (PFAS), short-chain fatty acids, bile acids, and phenolic metabolites.

Longitudinal analyses demonstrated high chemical stability and strong intra-individual reproducibility over time. At the same time, metabolic and exposure profiles were dynamically influenced by lifestyle-related factors, including physical activity, diet, and hormonal cycles.

To further evaluate the applicability of microsampling for dynamic metabolic studies, participants underwent a standardized dietary challenge consisting of a hamburger meal. Blood microsamples were collected before the meal and at regular postprandial intervals, and the experiment was repeated on three separate occasions. Postprandial metabolomic and exposomic responses showed good within-individual reproducibility, while substantial inter-individual variability was observed. Distinct response patterns were also associated with factors such as premeal exercise.

Overall, the study demonstrates that quantitative blood microsampling provides stable, analytically robust samples suitable for high-frequency longitudinal exposome and metabolomics investigations. Despite standardized dietary interventions, postprandial responses remained highly individualized, highlighting the personalized nature of metabolic regulation. Longitudinal profiling further revealed significant effects of lifestyle-related factors on both endogenous metabolic signatures and environmental exposure profiles.

These findings support microsampling as a powerful tool for personalized exposome and metabolomics research, enabling scalable real-world assessment of metabolic dynamics, environmental exposures, and lifestyle-associated variability over time.

Environmental chemical exposure reshape metabolic pathways linked to disease severity in metabolic dysfunction–associated steatotic liver disease

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Metabolic dysfunction–associated steatotic liver disease (MASLD) shows marked interindividual variability that cannot be explained by adiposity alone. Environmental exposures, particularly endocrine-disrupting chemicals such as per- and polyfluoroalkyl substances (PFAS), may contribute to this heterogeneity by altering metabolic pathways. Here, we applied an exposome–metabolomics approach to identify metabolic signatures linking chemical exposure to MASLD severity.

We performed an exposome-wide association study in 1,521 adults from the European Steatotic Liver Disease Registry. The samples were analysed using a comprehensive UHPLC-qToF-MS workflow covering lipids, semi-polar metabolites, bile acids, PFAS and other environmental chemicals. Associations with histological and non-invasive liver phenotypes were assessed using multivariable and sex-stratified models. Mechanistic support was obtained using PFAS exposure models in mice and HepaRG hepatocytes.

PFAS and parabens were associated with MASLD severity, including steatosis, inflammation, and fibrosis, as well as insulin resistance, in a sex-specific manner. PFAS exposure was linked to coordinated alterations in bile acids, lipid species (including phosphatidylcholines and sphingolipids), amino acids, and gut microbiota–derived metabolites, such as indole-derivatives. Bile acids were strongly associated with fibrosis (particularly in males), while extensive lipid remodeling was observed in both sexes. These alterations suggest a potential PFAS-driven modulation of gut microbiota composition or activity, supporting the hypothesis that disruption of the gut–liver axis may be an additional mechanism through which PFAS contribute to MASLD pathogenesis.

The perturbations observed in human plasma were directionally consistent with those detected in complementary in vivo and in vitro exposure models, providing mechanistic coherence and strengthening causal inference. Importantly, steatotic conditions combined with PFAS exposure produced substantially stronger metabolic disturbances than either PFAS exposure or steatosis alone.

Our findings identify reproducible metabolic signatures linking environmental chemical exposure to MASLD severity. Disruption of bile acid homeostasis, lipid metabolism, and gut–liver signaling pathways suggests that PFAS act as metabolic modulators of disease progression. This study highlights the value of integrating exposome and metabolomics data to uncover environmentally driven mechanisms in metabolic liver disease.

P12

Statin-Induced Metabolic Rewiring in Pancreatic Beta Cells: A ^{13}C -Glucose Tracing Study by Direct Infusion Mass Spectrometry

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Diabetic dyslipidemia affects 70–90% of adults with type 2 diabetes mellitus (T2DM) globally. Although statins impair beta-cell function, suppress insulin secretion, and elevate the risk of new-onset of T2DM in non-diabetic individuals, they remain the first-line pharmacotherapy for dyslipidemia. Despite widespread clinical use, the metabolic basis of statin-induced beta-cell dysfunction remains poorly defined. This gap limits rational risk stratification for statin therapy in at-risk populations and motivates the present investigation.

To address this critical knowledge gap, this study characterized atorvastatin-induced metabolic alterations in INS-1 beta cells under two T2DM-relevant stress models such as - glucotoxicity induced by high glucose and lipotoxicity induced by palmitic acid by utilizing ^{13}C -glucose isotope tracing and direct infusion high-resolution mass spectrometry (DI-HRMS).

Methodological optimization was initially performed on a high-resolution IQ-X Orbitrap mass spectrometer equipped with a direct infusion probe (DIP) by systematically screening four extraction solvents and four extraction strategies. A 2:3:3 water:acetonitrile:isopropanol solvent system paired with direct metabolite extraction from culture plates was found optimal for maximizing target metabolite coverage. INS-1 cells were subsequently exposed to glucotoxic or lipotoxic environments with or without atorvastatin treatment for short-term (10 minutes) and prolonged (24 hours) durations in the presence of $^{13}\text{C}_6$ -glucose as a stable isotope labeled metabolic tracer.

Resulting metabolomics datasets revealed that principal component analysis (PCA) achieved clear group separation between drug-treated and control cells exclusively in negative ionization mode, with separation becoming more pronounced at the 24-hour. Key drivers contributing to this negative-mode separation were identified as glycolysis and tricarboxylic acid (TCA) cycle intermediates. Isotope tracing further corroborated these findings by revealing deranged labeling patterns within central carbon metabolism, establishing it as the primary biochemical pathway disrupted by atorvastatin under diabetogenic stress conditions. these results demonstrate that atorvastatin exacerbates pre-existing cellular stress induced by high glucose or palmitic acid in INS-1 cells through central carbon metabolism. Future investigations should expand isotope tracing to additional labeled substrates (e.g., ^{13}C -palmitate, ^{13}C -glutamine) to completely resolve anaplerotic versus cataplerotic fluxes and validate the findings within primary pancreatic islets to significantly enhance translational clinical relevance.

P13/P14

Targeted Plasma Metabolomics Identifies Two Distinct Metabolic Responses to Acute Exercise in Breast Cancer Patients

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The aim of this study was to characterize acute exercise-induced changes in plasma metabolites of breast cancer (BC) patients before the start of therapy.

Methods

In total, 43 BC patient samples were analysed before and after an incremental exercise test. Blood plasma samples underwent metabolomics analysis using liquid chromatography–mass spectrometry. Altogether, 76 metabolites were quantified and statistical analysis was performed. In this study, we focused on energetic metabolites and nitrogen compounds, including amino acids. Patients were stratified into positive and negative inflammatory response groups based on exercise-induced interleukin-6 (IL-6) response. Paired statistical analysis and fold change analysis were performed to evaluate acute exercise-induced metabolic alterations.

Results

Distinct metabolic responses to acute exercise were observed between the two IL-6 response groups. In the IL-6 positive response group (n = 29), exercise induced significant increases in metabolites associated with glycolysis and tricarboxylic acid (TCA) cycle activity, including lactic acid ($p = 7.6 \times 10^{-6}$, FDR = 0.00054), malic acid ($p = 2.4 \times 10^{-5}$, FDR = 0.00085), and fumarate ($p = 7.5 \times 10^{-5}$, FDR = 0.00177). Significant decreases were also observed in several amino acids and nitrogen-related metabolites, including valine, isoleucine, phenylalanine, serine, and amino-n-butyric acid, suggesting increased amino acid utilization during exercise.

In contrast, the IL-6 negative response group (n = 9) demonstrated pronounced fold increases in ATP (fold change 3.74), L-cystathionine (fold change 2.88), and serotonin (fold change 2.04), indicating marked alterations in energy turnover, redox-related metabolism, and neuroendocrine signaling. However, these changes did not reach statistical significance, likely due to higher inter-individual variability and smaller sample size.

Overall, the findings indicate that breast cancer patients exhibit heterogeneous metabolic responses to acute exercise depending on inflammatory response profile. IL-6 positive responders demonstrated metabolic changes consistent with aerobic and anaerobic exercise response, whereas IL-6 negative responders showed a more variable stress-associated metabolic pattern. These results highlight the value of metabolomics in clinical and cancer research by enabling the identification of distinct metabolic phenotypes and revealing systemic biological responses that may not be detectable using conventional clinical biomarkers alone.

Mass spectrometry-based metabolomics as a key tool for biomarker discovery in pancreatic diseases

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Pancreatic diseases include inflammatory, neoplastic, and structural disorders. Acute pancreatitis (AP) is among the most frequent pancreatic diseases, while chronic pancreatitis (CP) represents a less common but progressive condition. Autoimmune pancreatitis (AIP) is considerably rarer. Pancreatic ductal adenocarcinoma (PDAC) is less frequent than inflammatory pancreatic diseases but has a disproportionately high mortality rate based on their late diagnosis [1]. Notably, inflammatory pancreatic diseases, particularly AIP and CP, are associated with an increased risk of PDAC, while AP may represent either a risk-associated inflammatory condition or an early clinical manifestation of occult pancreatic cancer [2,3]. In this context, the lack of biomarkers remains a major obstacle for early detection or prediction of its development.

This challenge is addressed by omics research, particularly mass spectrometry-based metabolomics, which provides sensitive and high-throughput analytical methods for biological sample screening. The analytical workflow is composed of complementary steps: sample preparation, ultra-performance liquid chromatography high resolution tandem mass spectrometry (UHPLC-HRMS/MS, Bruker maXis II ETD) analysis and data processing and statistical analysis. The data preprocessing pipeline applied R-packages such as XCMS, SERRF, and MSPrep. The multivariant and univariant methods were applied for discovering biomarker candidates. Annotation of features was performed via SIRIUS, HMDB and our in-house library.

Our metabolomics workflow was successfully applied to investigate pancreatic diseases across various biological matrices like serum, plasma, fecal or urine. The significant alternations in fecal metabolome were demonstrated for CP and AIP conditions [2]. Identified metabolites: enterolactone, 4-guanidinobutanonic acid, and methylthioadenosine sulfoxide were identified as a potential biomarker candidate to differentiate these two conditions. Furthermore, changes in metabolic pathways such as fatty acids, alkaloids, amino acids, and peptides underscore the gut metabolome's role in pathogenesis of these two inflammatory diseases. In other studies, the early-stage metabolomic alterations were discovered for high-risk PDAC individuals [3]. Plasma, urine and fecal metabolome showed significant candidates: methylglutaric acid, hydroxyindoleacetic acid and hypoxanthine, which are linked to pancreatic cyst. This research highlights promising approaches for biomarker identification within a systems biology framework.

P16

The SIMPLER Way to Measure Vitamin D₃ in Adipose Tissue: A High-Throughput LC–MS/MS Workflow

Per-Olof Bergh, **Ranjeet Kumar**, Jasmin Sebi, Rikard Landberg, Otto Savolainen

Vitamin D₃ is essential for human health and is stored in adipose tissue. Surprisingly little is known about the role of vitamin D₃ and how the adipose tissue concentrations relate to health because sampling and analysis are challenging. In this work, we developed a high throughput workflow to quantify vitamin D₃ in human small volume adipose tissue biopsies, combining a practical new sampling method with automated sample preparation and LC–MS/MS analysis.

We used samples from the SIMPLER clinical sub-cohort where adipose tissue had been collected from about 13 000 individuals using a vacuum assisted luer adapter system and kept at –80 °C until analysis. A centrifugation based approach was used to efficiently transfer the lipid material into 96 well plates, and sample mass was determined by weighing the tubes before and after transfer. To keep the method compatible with downstream lipidomics, saponification was omitted and a biphasic extraction (methyl tertbutyl ether:methanol, 3:1) was applied.

Sample preparation was semi-automated on an Opentrons Flex liquid handling platform, which will allow many samples to be processed from large cohorts (around 15,000 biopsies). After extraction and solid phase cleanup, vitamin D₃ was derivatized with PTAD to improve ionization and reduce matrix interference.

The method performed reliably, with good reproducibility (CV < 9%, n = 90) and high sensitivity (LLOQ of 0.06 ng/mL). Recovery was moderate but consistent at 45% and did therefore not affect analytical performance negatively.

Overall, this workflow offers a robust, scalable way to measure vitamin D₃ in small volume adipose tissue biopsies providing a solid foundation for large scale studies to reveal the role of vitamin D₃ levels in adipose tissue and its variation over time and their role in chronic disease.

P17

Non-targeted metabolomics reveals changes in plasma metabolome after an oat-rich low-gluten diet with candidate biomarkers of oat consumption

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Low-gluten diets (LGDs) are increasingly popular but may lack fiber that is essential for gut and overall health. Oats provide an important fiber source in LGDs and improve cholesterol status, yet the overall metabolic impact is unclear. The aim was to compare plasma metabolites of volunteers with increased cardiometabolic risk who followed either oat- or rice-rich LGD for 6 weeks, and to identify potential biomarkers of oat consumption.

Participants (n=69) were allocated in parallel groups of oat or rice and completed a 6-week randomized clinical trial. Rice, that is used in a similar way as oats in LGDs, acted as a control. Fasting plasma samples and dietary information were collected before and after the intervention. Plasma metabolites were analyzed with a non-targeted metabolomics approach using a high-resolution UHPLC-qTOF-MS platform with both positive and negative ionization modes and RP and HILIC chromatographies. Peak-picking was conducted with MS-DIAL, followed by data preprocessing with the notame package in R. Statistical significance was assessed with linear mixed-models and features of $q < 0.2$ were selected for annotation.

Metabolites showing time-treatment interactions during the oat diet included substantial increases in 2,6-dihydroxybenzoic acid and an unknown metabolite with m/z of 509.258, alongside moderate increases in 10 carbon medium-chain fatty acids, linoleic acid containing phosphatidylcholines and in primary bile acids, and decreases in 10-carbon medium-chain acylcarnitines with hydroxylated, saturated or unsaturated fatty acids. During the rice diet, trimethyluric acid, hexose monosaccharide, caffeine and its metabolites, several amino acids and dipeptides, and phosphatidylcholines with polyunsaturated fatty acids increased, and simultaneous decrease of circulating polyunsaturated fatty acids. The unknown metabolite 509.258 and 2,6-dihydroxybenzoic acid increased with oat intake and decreased with rice ($q < 0.05$), indicating a clear link with the oat-rich LGD.

Our study provides insights into the circulating metabolites after an oat-rich LGD in participants with increased cardiometabolic risk, reflecting endogenous metabolic responses to the dietary intervention, suggesting possible improvements in cardiometabolic health by alterations in lipid and energy metabolism. Additionally, we identified candidate plasma biomarkers of oat consumption contributing to the on-going research on cereal biomarkers.

Exploring Metabolite-Based Cluster Patterns Associated with Severity in Inflammatory Bowel Disease

Cezar Fabian Moise, Malene Revsbech Christiansen, Marie Vibeke Vestergaard, Tine Jess, Filip Ottosson

Background: Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), is a chronic immune-mediated condition with a heterogeneous clinical course that is increasingly being explored through advances in omics, particularly metabolomics. IBD metabolomic studies have revealed dysregulation in several molecular pathways across different disease phases [1], yet associations between the metabolite landscape and disease severity have not been thoroughly explored. This study examines whether serum metabolite profiles from patients sampled two years before to one year after diagnosis are associated with disease severity.

Methodology: This study leveraged the nationwide PREDICT cohort linked to the Danish National Biobank, with IBD defined through the Danish National Patient Register. Untargeted metabolomics was performed using liquid chromatography-mass spectrometry (LC-MS), and metabolites were annotated using GNPS-based molecular networking, SIRIUS+CSI:FingerID, and CANOPUS. Metabolite abundance data were interrogated using dimensionality reduction methods. Unsupervised clustering was subsequently applied on non-linear metabolite projections obtained through dimensionality reduction, followed by exploratory machine learning across five algorithms. The most relevant clusters from the best-performing severity classification models were independently validated using the Boruta algorithm prior to biological interpretation.

Results: Metabolite data showed clear non-linear trends following dimensionality reduction. Unsupervised clustering resulted in 60 stable metabolite clusters comprising 2458 metabolites. The strongest performance in severity classification was achieved by the Random Forest classifier in CD cases. Notably, molecules such as hippurate and indole-propionic acid, as well as secondary bile acids and gamma-glutamyl-containing peptides, were associated with non-severe cases, whereas glycerophosphocholines were linked to severe CD but non-severe UC. Our findings highlight the need for subtype-specific modeling approaches and point to the potential of metabolomics as a tool for risk stratification in IBD, as well as for identifying biological pathways that may contribute to disease severity and progression.

Conclusion: We developed a novel framework linking metabolomic profiles to IBD severity, with stronger classification performance observed in CD via Random Forest. Distinct metabolite cluster associations were identified for CD and UC severity respectively. This work highlights the potential of metabolite clusters in offering information about disease severity trajectory while underscoring the need for multi-omics integration and mechanistic studies to realistically elucidate how metabolite networks shape disease outcome.

[1] Bjerrum et al. (2021), eBioMedicine, <https://doi.org/10.1016/j.ebiom.2021.103551>.

Exploring Oral Microbiome–Metabolome Associations in Alström and Bardet–Biedl Syndromes Using Complementary Multi-Omics Approaches

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Background: Alström syndrome (ALMS) and Bardet–Biedl syndrome (BBS) are rare ciliopathies characterised by multisystem involvement, including obesity, insulin resistance, and type 2 diabetes. Systemic metabolic alterations may be accompanied by changes in the oral microbiome and metabolome; however, relationships between these molecular layers in ALMS and BBS remain poorly understood.

Methods: Saliva and gingival crevicular fluid (GCF) samples were collected from individuals with genetically confirmed ALMS and BBS, as well as from obesity and healthy control groups. Oral microbial communities were profiled using V3–V4 16S rRNA gene amplicon sequencing, and untargeted metabolomic profiling of saliva and GCF was performed using gas chromatography–mass spectrometry (GC–MS). Microbiome–metabolome associations were assessed using Spearman’s rank correlation analysis. Complementary multi-omics integration was performed using unsupervised Multiple Co-Inertia Analysis (MCIA) and the supervised DIABLO framework (mixOmics) to investigate shared variation and coordinated patterns across the oral microbiome, salivary metabolome, and GCF metabolome.

Results: Spearman’s correlation analysis revealed multiple associations between oral bacterial genera and metabolites in both GCF and saliva, primarily involving amino acids, organic acids, fatty acids, and carbohydrate-related metabolites. The ALMS+BBS group exhibited distinct patterns of positive and negative microbiome–metabolome correlations compared with the obesity and healthy control groups. Several associations involved bacterial genera previously reported to differ in the ALMS+BBS group, including *Prevotella*, *Enterococcus*, *Eikenella*, *Capnocytophaga*, and *Lachnoanaerobaculum*, together with metabolites such as 3-hydroxybutyric acid, amino acids, and carbohydrate-related compounds. MCIA identified shared variation across all three omics layers, with the first two components accounting for approximately 42% of the cumulative inertia and showing partial separation of the study groups. DIABLO further identified coordinated multi-omics patterns involving *Prevotella*, *Enterococcus*, *Lachnoanaerobaculum*, and *Lactobacillus*, together with 3-hydroxybutyric acid, urea, valine, alanine, and α -D-glucosamine phosphate. Group separation based on the first two DIABLO latent components was supported by PERMANOVA across the microbiome, salivary metabolome, and GCF metabolome data blocks (all $p < 0.001$).

Conclusions: Combined correlation and multi-omics analyses revealed coordinated relationships between oral microbial composition and metabolic profiles in ALMS and BBS. Complementary Spearman, MCIA, and DIABLO analyses identified recurring microbial and metabolic features across saliva and GCF, suggesting that these rare ciliopathies are associated with distinct oral microbiome–metabolome patterns. These exploratory findings provide a basis for further studies in larger, independent cohorts to determine their biological and potential clinical relevance.

NIST-Guided Evaluation of Reverse-Phase Columns for Integrated Exposomics and Metabolomics Profiling

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The human exposome encompasses a wide range of exogenous and endogenous compounds, including xenobiotics, phytochemicals, microbial metabolites, and endogenous metabolites. Interactions between exogenous and endogenous molecules are increasingly recognized, and integrated exposomics–metabolomics workflows have been developed to capture these complex chemical profiles within a single analysis. However, reverse-phase (RP) column performance can vary depending on sample matrix, analytical focus, and study scale, influencing both metabolite coverage and quantitative reproducibility. Guidance on RP column selection for specific integrated exposomics/metabolomics applications remains limited.

This study provides a comparative evaluation of RP-LC columns for integrated exposomics and metabolomics analysis. Three RP columns, BEH-C18 50 mm (BEH-5), BEH-C18 100 mm (BEH-10), and CSH-C18 100 mm (CSH-10), were evaluated using four NIST reference materials: serum (SRM 1957), plasma (SRM 1950), vegan feces (RGTM Stool Vegan 10162), and omnivore feces (RGTM Stool Omnivore 10172). Samples were extracted using the SIMPLIFY integrated exposomics/metabolomics workflow and analyzed by untargeted LC–HRMS on a Waters Xevo G3-QTOF in negative ionization mode. Compound annotation was performed using an in-house library (~1000 compounds) together with public MS/MS spectral databases, covering xenobiotics, phytochemicals, gut microbial metabolites, and endogenous metabolites.

Across all matrices, 594 metabolites were identified, spanning four biochemical source categories and nine chemical superclasses. Among the evaluated columns, BEH-10 showed the highest overall metabolite coverage, whereas CSH-10 demonstrated improved performance for phytochemicals and amino acids. BEH-5 enabled faster analysis but resulted in a 15-20% reduction in metabolite coverage relative to the 100 mm columns. Matrix-dependent differences revealed distinct chemical profiles, with fecal samples enriched in phytochemicals and gut microbial metabolites compared with blood-derived matrices, alongside clear compositional differences between vegan and omnivore fecal samples.

The combined use of blood- and feces-based NIST reference materials enabled cross-matrix performance assessment through overlapping metabolite coverage, supporting harmonization and quality assurance strategies for multi-matrix exposomics/metabolomics studies. Overall, this work provides a NIST-guided evaluation framework for improving reproducibility, comparability, and column selection in RP-LC–HRMS-based integrated exposomics and metabolomics workflows.

P21

Computational Metabolomics of Psychotropic Alkaloids: From Large Chemical Space to Neurodegenerative Disease Targets

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Natural products represent a vast and chemically diverse reservoir of neuroactive compounds, yet navigating alkaloid chemical space for neurodegenerative disease discovery remains a major challenge. Within the NeuroHEALTH MSCA project, we developed an integrated computational metabolomics workflow to accelerate the discovery and prioritization of neuroactive alkaloids targeting key proteins involved in neurodegeneration. Initial large-scale data mining of publicly available resources, including LOTUS, ChEMBL, NPAtlas, and literature sources, yielded a library comprising more than 10,000 alkaloid structures. To efficiently navigate this large chemical space, computational prioritization workflows integrating molecular docking, cheminformatics, metabolomics-informed filtering, and drug-likeness criteria based on Lipinski's rules were applied against multiple protein targets associated with Alzheimer's and Parkinson's disease. The data mining and prioritization pipeline was automated through KNIME and RDKit workflows integrating tools such as AutoDock Vina and RxDock, enabling scalable and reproducible analysis. Top-ranked compounds and prioritized plant and fungal species were subsequently selected for experimental investigation following the computational prioritization workflow. Raw biological material was collected and subjected to phytochemical extraction and LC–MS/MS-based untargeted metabolomics analysis, linking in silico predictions with experimental chemical profiling. Alkaloid-rich extracts were analyzed using GNPS-based molecular networking combined with in silico annotation tools including SIRIUS and CANOPUS to characterize alkaloid chemical space and prioritize biologically relevant metabolites. Prioritized compounds and fractions were subsequently purified and evaluated through in vitro assays and in vivo zebrafish models to assess toxicity and neuroprotective activity against neurodegenerative disease-related phenotypes. Preliminary analyses highlight the enrichment of specific alkaloid subclasses, including aporphine- and mesembrine-type alkaloids, as promising neuroactive scaffolds. Overall, the integration of AI-assisted data mining, computational prioritization, and LC–MS/MS-based metabolomics demonstrates a scalable workflow for navigating large natural product chemical spaces, accelerating neuroactive compound discovery, and supporting the transition from psychotropic alkaloids to novel neurodegenerative disease therapeutic agents.

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P22

Lipidomic Biomarkers for Mild Traumatic Brain Injury: Severity Assessment, Outcome Prediction, and Longitudinal Monitoring

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Traumatic brain injury (TBI) causes long-term disability with significant societal burden. It is commonly classified as mild, moderate, or severe using the Glasgow Coma Scale; however, better phenotypic characterization is needed to improve patient management and outcome prediction. Serum lipid profile alterations have emerged as candidate prognostic markers reflecting TBI pathophysiology. We analyzed serum lipid profiles from 929 mild TBI patients enrolled in CENTER-TBI, a large European multicenter cohort, with 6-month outcomes assessed by the Glasgow Outcome Scale–Extended (GOS_E). Serum samples collected within 24 hours of admission were profiled by UHPLC-MS, using class-specific lipid standards for quantification. Lipid and protein data were integrated using ordinal elastic-net regression and validated by proportional odds logistic regression. Models were adjusted for variables in the CRASH clinical outcome prediction model and validated in an independent test set. Compared with the clinical baseline model, combined models achieved AUCs of 0.702–0.802, with key contributions from lipids LPC(18:2), LPC(20:5), and PC(O-34:3), and proteins GFAP, NFL, and S100B. In a longitudinal sub-cohort from CENTER-TBI, multi-omics factor analysis (MOFA2) was applied to identify latent factors summarizing coordinated lipid co-abundance patterns across 2,126 samples from 632 subjects in different hospital strata spanning seven post-injury timepoints. Factor scores were subsequently associated with neurological outcomes using proportional odds ordinal logistic regression. Taken together, serum lipidomics integrated with protein biomarkers demonstrates strong potential for outcome prediction in mild TBI, addressing a significant unmet clinical need.

P23

Sahlgrenska Clinical Urine Metabolomics Platform for Precision Diagnosis of Inborn Errors of Metabolism

Henrik Ryberg, Dimitra Lappa, Sara Ahmed, Parisa Emamy, Fredrik Sterky, Ann-Charlotte Almstrand, Moa Andresen Bergström, and, Yan Shen

Inborn errors of metabolism (IEMs) are a group of approximately 1,500 inherited disorders caused by defects in metabolic pathways. Early and accurate diagnosis is essential to prevent irreversible damage and improve clinical outcomes. Genomic approaches have significantly advanced molecular diagnostics, but biochemical analyses remain indispensable for early detection, interpretation of genetic variants, and monitoring of treatment. However, current laboratory approaches for diagnosis of IEMs are largely limited to targeted assays that are often laborious and may not capture the full complexity of metabolic disturbances.

In this project, we aim to establish a clinically metabolomics platform based on liquid chromatography–Orbitrap mass spectrometry (LC-HRMS), integrated with bioinformatics and machine learning for clinical interpretation. In a first aim, the analytical platform will be designed for targeted quantification of approximately 120 clinically relevant metabolite biomarkers while simultaneously allowing the acquisition of thousands of additional metabolic features, enabling both targeted and untargeted analysis within a single workflow.

The LC-HRMS assay is currently under development at the Department of Clinical Chemistry, Sahlgrenska University Hospital, Sweden. A comprehensive set of calibrators and reference standards has been developed and tested, forming the basis for quantitative analysis. The next phase will focus on clinical validation of the assay, including evaluation of sensitivity, specificity, reproducibility, and clinical performance, with the goal of implementation in routine diagnostics.

Building upon the analytical workflow, dedicated downstream bioinformatic pipelines are being developed for both targeted quantitative and untargeted high-dimensional metabolomics profiling. Workflows include peak detection, retention-time alignment, normalization, multi-level quality control, along with statistical strategies to address batch effects, technical variation and complex missing data structures (MNAR/MAR). Machine learning approaches will be deployed to facilitate pattern recognition and prioritization of clinically relevant metabolic deviations at the individual patient level. In addition, the project will explore data-driven approaches for establishing clinically applicable reference intervals and integrating metabolomics findings with biochemical pathways databases. The pipelines aim to support biologically informed clinical interpretation of metabolic disturbances within a clinically supervised framework to link metabolic findings to biological pathways and disease mechanisms.

The platform will be validated by using clinical cohorts of patients with confirmed IEMs. By integrating robust analytical methods with advanced bioinformatics, this work aims to transition metabolomics from a research tool to a clinically implemented diagnostic approach. Ultimately, the project has the potential to significantly shorten diagnostic timelines, increase diagnostic yield, and advance precision medicine by enabling more tailored, individualized patient care.

P24

N-acetylcysteine induces arginine biosynthesis in *Staphylococcus aureus* without impairing growth

Theresa Schiemer, Claudia Siverino, Vimalnath Nambiar, Kristaps Klavins

N-acetylcysteine (NAC) is increasingly investigated as an antibacterial agent against *S. aureus*. But reported minimum inhibitory concentrations vary more than 50-fold across studies – a discrepancy we attribute to inconsistent reporting on the final pH. NAC is a weak organic acid, and we demonstrated that antibacterial activity is strictly pH dependent when tested on two different *S. aureus* strains. At neutral pH, NAC had no effect on bacterial growth.

Despite the absence of growth inhibition, pH-neutral NAC induced concentration-dependent metabolic reprogramming (0-200 mM) under both planktonic and biofilm conditions, quantified by targeted LC-MS measurement. In the tested clinical isolate, the dominant response was a significant accumulation of arginine both intra- and extracellularly, consistent with NAC-triggered arginine biosynthesis – a capacity usually absent in *S. aureus* but often emerging in clinical isolates. This was accompanied by cysteine accumulation, decreased TCA cycle intermediates and increased lactic acid production, suggesting a shift from oxidative to fermentative metabolism. These metabolic changes partially overlapped between planktonic and biofilm bacteria despite their distinct baseline metabolic states.

Our findings reframe NAC as a metabolic modulator of *S. aureus* under physiological pH rather than a direct antibacterial agent, with potential implications for antibiotic tolerance and virulence modulation.

Deep multimodal metabolomic phenotyping of cerebrospinal fluid from almost 400 patients with cognitive and motoric neurological disorders

Jannik Sprengel, Sajitha Sadasivan, Vaishnavi Chidambaram, Claire Wegner, Sven W. Meyer, Matthew R. Lewis, Christoph Trautwein

Cerebrospinal fluid (CSF) is an important diagnostic matrix which is being used in the clinical environment for diagnosis of acute or latent neurological conditions and disorders. The immediate relation of this biofluid to the brain tissue provides a unique reservoir to measure lipids and small molecules that may directly indicate and/or affect damages and diseases of the central nervous system (1, 2). However, the highly invasive extraction procedure for CSF limits the availability of these valuable samples, especially healthy controls.

In this work we analyzed a large cohort of CSF samples from 396 patients and donors, which were separated into seven groups according to neurological diagnosis: Alzheimer's disease (AD; n=77), amyotrophic lateral sclerosis (ALS; n=36), frontotemporal dementia (FTD; n=62), mild cognitive impairment (MCI; n=87), Parkinson's disease (PD; n=36), progressive supranuclear palsy (PSP; n=75) and healthy controls (n=23). All samples were analyzed via multimodal untargeted metabolomics, integrating both RP and HILIC LC-TIMS-MS data (timsTOF Pro 2 and timsMetabo platforms) with 600 MHz IVDr ¹H-NMR data. Additionally, untargeted lipidomics analysis was performed on the timsTOF Pro 2 system. Annotation of the LC-MS data was performed using both in-house references and commercially/publicly available libraries. NMR-based metabolite annotation was performed through Bruker's Advanced Chemical Profiling (ACP 2.0) software.

The combination of these techniques allowed the annotation of several hundred metabolites and lipids. Statistical analysis revealed significant differences for several compounds between patient groups. For example, HILIC-TIMS-MS analysis revealed highly increased signals of isoferulic acid in patients with motoric disorders, especially PD and PSP, as well as significantly reduced levels of bis(p-methylbenzylidene)sorbitol across neurological patients compared to the healthy control group. Meanwhile, RP-LC-TIMS-MS showed an upregulation of octanoyl-carnitine in patients from the AD, MCI and PD groups versus the healthy controls. These findings showcase the great potential of expanding the metabolite coverage through multimodal metabolomics, using combinatory approaches of LC-MS and IVDr NMR, to identify potential diagnostic biomarkers that can be further developed to predictive markers, using follow-up validation cohorts.

Untargeted Metabolomics for the Identification of Common Pan-Cancer Metabolic Signatures across Lung, Colorectal, and Ovarian Cancers

Efstathia Vouzaxaki, Ioanna Tsiara, Tobias Sjöblom, Daniel Globisch

Although lung, colorectal, and ovarian cancers arise from different underlying causes, they often affect the body's metabolism in similar ways. This study aimed to characterize plasma metabolomic dysregulations across four clinical stages (I–IV) to identify both pan-cancer bio-fingerprints and disease-specific diagnostic biomarker panels.

Plasma samples (N = 710, including 615 cancer patients across stages I–IV of lung, colorectal, and ovarian malignancies, alongside 95 non-cancer controls) were randomized into three independent analytical batches to minimize systematic analytical bias. High-resolution untargeted profiling was executed on a UHPLC system coupled to a Maxis II ETD Q-TOF mass spectrometer equipped with an electrospray ionization (ESI) source. Chromatographic separation was achieved using an Acquity UPLC HSS T3 column. Data acquisition was performed in AutoMSMS mode (data-dependent acquisition, m/z = 50–1,200) across both positive and negative ionization polarities.

Raw files were processed using the XCMS software suite for automated feature detection, peak picking, and retention-time alignment. To address within-batch instrument drift, signal attenuation was normalized via Systematic Error Removal using Random Forest (SERRF) calibrated against pooled quality control (QC) samples, followed by multi-batch integration utilizing the ComBat algorithm. Feature matrices were subjected to univariate statistical evaluation (Student's t-test and ANOVA with Tukey's HSD post-hoc correction) and unsupervised principal component analysis (PCA). Multi-marker predictive panels were optimized using logistic regression models (liblinear solver) with bootstrap resampling (n = 1,000 iterations) and ranked according to the Area Under the Receiver Operating Characteristic Curve (AUC-ROC). Structural annotation of candidate metabolites (confidence level ≥ 2) was confirmed through tandem MS/MS fragmentation pattern matching against public and in-house spectral libraries.

Supervised multivariate classification yielded distinct separation between oncology cohorts and non-cancer controls, with multi-marker diagnostic panels achieving consensus AUC-ROC values up to 0.95. Stage-dependent differential expression highlighted progressive dysregulation in phospholipid remodeling, alongside alterations in Phase II xenobiotic biotransformation and organo-sulfur pathways. Shared metabolic features reflected heightened oxidative stress, systemic immunometabolic reprogramming, and altered amino acid catabolism, whereas tissue-specific metabolite profiles correlated with organ microenvironment dynamics, exogenous exposures, and gut microbiome-host co-metabolism.

P27

Metabolomic signatures associated with development of late effects in gynecological cancer survivors in the LETSGO trial

Feng Wang, Guro F. Giskeødegård, Julia Debik, Liv Cecilie Vestrheim Thomsen, Line Bjørge, Tone F. Bathen, Ingvild Vistad

Background

Advances in gynecological cancer treatment have improved survival, yet approximately 30% of survivors experience symptoms that significantly impair quality of life.^{1, 2} Consequently, patient care increasingly aims to preserve and enhance quality of life. However, no established methods exist to predict treatment-related symptom development. Late effects are defined as adverse effects occurring after completion of cancer therapy.³ Metabolomics offers a promising approach to capture metabolic and physiological alterations of pathobiological relevance and may enable identification of clinically useful biomarkers.

Aims: To identify metabolic patterns associated with therapy-related late effects in gynecologic cancer survivors and to evaluate whether early posttreatment metabolomic signatures can predict the development or persistence of these effects.

Methods: We will analyze phenotypic data and biological samples from the LETSGO (Lifestyle and Empowerment Techniques in Survivorship of Gynecologic Oncology) trial.⁴ LETSGO is a multicenter cohort study including 741 patients treated for gynecological cancers across 12 Norwegian hospitals (REK sør-øst 2019/314). Blood samples were obtained from 726 patients at 3, 12, and 36 months post-treatment, or at recurrence if this occurred during the followup period. Plasma metabolomic profiles will be assessed using high-resolution nuclear magnetic resonance (NMR) spectroscopy. Approximately 50 small-molecule metabolites, 112 lipoprotein subfractions, and 4 inflammatory markers will be quantified using the commercially available Bruker IVDr platform. Patient reported outcome measures (PROMs) will be used to assess quality of life, symptom burden, and functional status, with scoring performed according to established guidelines. Longitudinal metabolomic data will be analyzed using multivariate approaches, including repeated measures ANOVA-simultaneous component analysis (RM-ASCA+), penalized regression and network analysis, to identify metabolomic signatures associated with late effects and lifestyle factors.

Conclusions

This project may provide novel insights into the metabolomic heterogeneity of gynecological cancer survivors and its relationship to late effects and quality of life. Identification of metabolomic patterns at 3 months post-treatment that predict late outcomes could support risk stratification tools and inform targeted survivorship interventions.

Plasma Metabolomics Differentiates Cardiometabolic Disease Progression from Endurance-Associated Lipid Remodeling

Pramod P. Wangikar, Sneha Rana, Nikita Savalsang, Avinash Vellore Sunder, Deepti Sahasrabuddhe

Introduction: The cardiovascular–kidney–metabolic axis defines a complex continuum of cardiometabolic risk, yet molecular signatures distinguishing pathological progression from beneficial adaptation remain poorly defined. We applied untargeted plasma metabolomics and lipidomics across two complementary cohorts: progressive cardiometabolic disease spanning type 2 diabetes (T2DM), diabetic kidney disease (DKD), and diabetic cardiovascular disease (DCD), as well as endurance-trained marathon runners as a model of physiological adaptation, both anchored against healthy sedentary controls. This design allowed us to map the metabolic landscape from disease burden to peak fitness, identifying circulating signatures for disease staging, risk stratification, and mechanistic contrast between pathological and adaptive remodeling.

Methods: Plasma samples were collected from Asian Indian participants with T2DM (n=60), DKD (n=39), DCD (n=50), and non-diabetic sedentary controls (n=81). Marathon runners (n=36) were sampled at baseline, immediately post-race, and 72 h post-race to capture chronic adaptation and acute metabolic responses. Metabolites were profiled by untargeted LC–MS and GC–MS, and an in-house AI/ML-enabled workflow integrating batch correction, multivariate modeling, and pathway-level interpretation was applied uniformly across both cohorts.

Preliminary Data: Across the disease-to-adaptation spectrum (Runners → Controls → T2DM → DKD → DCD), metabolomic features converged into three interconnected yet mechanistically distinct remodeling patterns:

- **Kidney-associated signatures:** Progression to DKD was marked by monotonic accumulation of uremic and gut-derived metabolites including indoxyl sulfate, p-cresol sulfate, and phenylacetylglutamine, conspicuously absent in runners and controls, anchoring these signals to renal dysfunction. Notably, uremic signatures enabled disease staging independently of lipid alterations seen in DCD, highlighting the organ-specific nature of metabolic deterioration.
- **Cardiovascular-associated lipid remodeling:** DCD revealed a complementary lipid phenotype with selective LPC depletion coupled with accumulation of medium- and long-chain acylcarnitines, reflecting inflammatory lipid remodeling and impaired mitochondrial β -oxidation. This pattern clearly differentiated DCD from DKD and T2DM, underscoring that cardiovascular and renal complications leave biochemically separable imprints on the circulating metabolome.
- **Endurance-associated metabolic remodeling:** In striking contrast, marathon runners showed no uremic burden and instead displayed baseline enrichment of PUFA-containing LPC species with reduced short-chain acylcarnitines, consistent with favorable membrane lipid composition and preserved mitochondrial efficiency. Acute post-race perturbations resolved within 72 h, while inter-individual heterogeneity in baseline LPC profiles reflected variable degrees of long-term adaptation.

Cross-Cohort Integration: Unified analysis reveals a coherent molecular narrative: uremic metabolites track renal deterioration, phospholipid depletion signals cardiovascular compromise, and PUFA-LPC enrichment marks adaptive physiology. Crucially, LPC composition emerges as a shared molecular axis connecting pathological loss in DCD and physiological gain in endurance, offering a novel lens through which disease and health can be simultaneously interrogated.

Novel Aspect: By integrating disease progression and physiological adaptation within a single framework, this study disentangles renal, cardiovascular, and endurance-associated metabolomic remodeling, positioning LPC composition as a key discriminator between cardiometabolic pathology and adaptive physiology.

P29

An Open-Access Atlas Integrating a Decade of Metabolomics Research in Endometrial Cancer

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Endometrial cancer (EC) is one of the most common gynecological malignancies worldwide, with a steadily increasing incidence. Metabolomics has revealed numerous EC-associated metabolic alterations and holds promise for improving disease characterization and biomarker discovery. However, these findings remain scattered across studies employing different biological matrices, analytical platforms, clinical comparisons, and metabolite nomenclature, limiting their direct comparison and broader interpretation. Therefore, we developed an open-access Endometrial Cancer Metabolite Atlas to consolidate and harmonize the available metabolomics evidence and provide an accessible resource for EC research.

PubMed was searched for human EC metabolomics studies using combinations of the terms “endometrial cancer”, “endometrium carcinoma”, or “uterine cancer” with “metabolomics”. The present release covers publications from 2014-2024. Thirty-six studies met predefined inclusion criteria requiring information on the study population, biological sample type, and analytical technique. Metabolites reported by the original studies as statistically significant were curated together with the reported direction of the between-group difference, biological matrix, analytical platform, study design, and clinical comparison. Records were further annotated with molecular formula, monoisotopic mass, metabolite class and subclass, and identifiers from HMDB, KEGG, LIPID MAPS, and PubChem.

The Atlas currently comprises more than 2,200 metabolite–study records from plasma, serum, urine, tissue, and cervicovaginal fluid studies using LC-MS, GC-MS, or NMR. It integrates findings across diverse clinical comparisons, including EC versus healthy controls or hyperplasia, type I versus type II EC, early versus advanced disease, low- versus high-grade and low- versus high-risk groups, obesity-related subgroups, and pre- versus postoperative states. The interactive web application allows users to search, filter, and compare reported metabolic alterations while retaining their analytical and clinical context.

By transforming dispersed literature findings into a structured, openly accessible knowledge resource, the Endometrial Cancer Metabolite Atlas enables cross-study exploration of EC-associated metabolic alterations and may help identify repeatedly reported findings, inconsistencies, and knowledge gaps. It may therefore support the interpretation and design of future clinical metabolomics and biomarker studies in EC. Designed as an evolving resource, it will be expanded to incorporate newly published studies, including those from 2025 onward.

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MALDI-mass spectrometry imaging as a tool for Mapping Lesion Architecture in a Mouse Model of Multiple Sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease that primarily affects young adults between 20 and 30 years of age. An autoimmune response produces focal lesions characterized by demyelination and the formation of glial scars in the brain. In addition to these lesions, early diffuse inflammation causes damage and atrophy in both grey and white matter. Recent evidence indicates that presence of characteristic broad rims around chronic MS lesions is associated with more aggressive disease and may predict progression. The prognostic use of rim features is promising but still not fully described. In this study, we used spatial lipidomics to map the heterogeneity across lesion zones, aiming to identify specific lipid signatures that distinguish rims and lesion cores in the mouse striatum after experimentally induced MS-like lesions. Using C57BL/6 mice (3 months old) with a well-defined neuroinflammatory profile, we combined immune priming and stereotactic lesion induction to model localized inflammation as in MS. Mice received an emulsion of myelin oligodendrocyte glycoprotein (MOG) peptide for immune activation, followed by targeted injection of heat-killed *Mycobacterium tuberculosis* into the left striatum; sham controls received saline. Untargeted lipidomics was performed using Bruker timsTOF fleX mass spectrometer equipped with MALDI ion source and MALDI-2 post ionization at 20 μm spatial resolution. Lesion formation was confirmed using H&E staining. Key lipid species, including ceramides, phosphatidylcholines (PCs), and phosphatidylethanolamines (PEs), were found to be concentrated at the lesion site compared to sham controls.

Compared to H&E staining, the lipidomics profile showed greater spatial differentiation between zones inside and outside of the lesion. Moreover, lesion-associated lipid features were often present in brain vasculature, indicating their involvement in peripheral responses. These preliminary findings highlight the importance of lipid research and monitoring in neurological diseases. Lipids are a promising marker of neuronal damage and inflammation, involved in the local responses but also in coordinating systemic or peripheral responses.

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Tailoring the extraction: Advanced solvent strategies and Nitrogen Nanobubbles for spatial metabolomics in PA nano-DESI

Sireethorn Poomborplab, Ingela Lanekoff

Pneumatically assisted nanospray desorption electrospray ionisation (PA nano-DESI) is a powerful ambient ionisation technique in mass spectrometry that enables the continuous extraction and analysis of biomolecules directly from a sample surface. The sampling system consists of two fused-silica capillaries aligned at approximately 90°. The primary capillary continuously supplies the extraction solvent, forming a dynamic liquid bridge on the sample surface. Desorbed analytes are extracted and transferred through the secondary capillary, where a high-pressure tee introduces nitrogen gas to assist analyte transportation and electrospray ionisation (ESI) into the mass spectrometer.

By continuously scanning the probe across a tissue section, this technique facilitates high-resolution spatial metabolomics and lipidomics studies. However, mapping the spatial distribution of analytically challenging molecules, such as low-abundance metabolites or compounds that are difficult to ionise, remains a major challenge for ambient mass spectrometry imaging techniques. To overcome this, rigorous method optimisation is required to tailor the extraction environment to the target analytes.

PA nano-DESI allows extraction solvent optimisation, making it a highly versatile imaging tool. In this work, we explore current strategies for extraction solvent optimisation to improve the detectability of biologically significant metabolites and lipids. Specifically, we demonstrate how altering organic solvent proportions and adding chemical additives directly enhance metabolite and lipid signal intensity across tissue sections. Moreover, we introduce a novel extraction solvent preparation method: nitrogen nanobubble-infused solvent, highlighting its potential to enhance overall analytical sensitivity for complex biological tissue.

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Expanding the chemical space in shotgun lipidomics by selective liquid-liquid extraction

Varun V. Sharma, Ingela Lanekoff

Shotgun lipidomics provides rapid profiling of complex samples but is often limited in chemical space, leaving low-abundant and poorly ionizable species, such as steroids and oxidized neutral lipids, underrepresented.¹ Although silver adduction can enhance detection of these molecular species, it poses a challenge because silver precipitates as chloride rapidly in biological samples. Additionally, detection of steroids and oxidised neutral lipids is often limited by more abundant lipids such as phospholipids. Here, we have overcome these challenges and present a modular, silver-compatible extraction workflow with reduced interferences from phospholipids. The method is based on dry MTBE extraction and pre-evaporation addition of Ag⁺, and we show that this minimizes co-extraction of abundant lipids and enables formation of stable silver-lipid complexes, with significantly enhanced signal intensity. When combined with a direct infusion probe, we report rapid analysis of low sample volumes with high signal intensity compared to traditional extraction strategies such as Matyash.² Using this workflow, we profiled six 3-hydroxy-5-ene steroids from newborn calf serum and characterized oxidized cholesteryl esters, mono-, di-, and triacylglycerols. Furthermore, we show that silver adduct fragmentation provides structural specificity, including hydroxyl position determination and identification of cholesteryl-prostaglandin esters. Overall, our method “LLE-Select” extends the chemical space accessible to shotgun lipidomics and offers a rapid, sensitive, and flexible platform for mass spectrometric detection of challenging lipid classes.

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Fecal SCFA dynamics reflect trophic-level succession in mammalian gut microbiota

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Short-chain fatty acids (SCFAs) are key metabolites produced by the gut microbiota and serve as important functional readouts of the gut microbiome activity. In early life, they have been linked to immune development, colonization resistance against pathogens, and metabolic health. However, robust and transferable analytical workflows for SCFA profiling, including their metabolic intermediates, remain limited across laboratories and biological systems.

Here, we present a targeted liquid chromatography–mass spectrometry (LC–MS) workflow based on 3-nitrophenylhydrazine derivatization for the simultaneous quantification of 15 SCFAs and 8 related organic acids. The method was analytically validated across two independent laboratories using distinct LC–MS platforms, which demonstrated reproducibility and inter-laboratory applicability. We then applied the workflow to fecal samples from multiple biological systems, including gnotobiotic mouse models with different gut microbial colonization states, piglets, and 197 humans spanning infancy to adulthood.

Integrating metabolite profiles across colonization states, host species, and human age groups identified conserved metabolic trajectories associated with gut microbiota maturation. Early-life and simplified microbiota ecosystems enriched in *Bifidobacterium* spp. were characterized by lactate dominated profiles. In contrast, increasing microbial complexity was associated with elevated levels of particularly butyrate and propionate across hosts and colonization models, which suggested conserved patterns of microbiota metabolic development. Further, segmented regression analysis in the human cohort identified a distinct metabolic transition at three years of age, indicating a developmental shift in the microbiota functionality. These findings support trophic-level succession and cross-feeding networks as core principles of gut microbiota metabolic development.

Taken together, this work established a transferable targeted metabolomics workflow for SCFA and related organic acid quantification. Additionally, it provides reference ranges, conserved transitions and biological context for interpreting microbiome function across human age and development.

P34

Chiral octadecanoid profiling reveals inflammatory signatures of the scalp microbiome

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Octadecanoids are C18 fatty acid-derived oxygenated lipids that are involved in numerous mammalian biological processes, including inflammation, nociception, and cell proliferation. The onset and development of skin diseases such as psoriasis or seborrheic dermatitis are influenced by octadecanoids and their complex array of pro- and anti-inflammatory actions (1). Octadecanoid levels at the skin surface could depend on its microbiome, with some skin-resident yeasts suspected to metabolize sebaceous lipids into pro-inflammatory octadecanoids (2).

A two-step approach was used to evaluate this hypothesis: 1) The scalp of 21 subjects was washed with ethanol to collect scalp surface lipids, before and after a 5-day treatment with antifungal shampoo, while the associated scalp microbial DNA was collected with swabs and subject to qPCR. 2) The 4 main scalp microbes (*Malassezia restricta*, *Malassezia globosa*, *Cutibacterium acnes*, and *Staphylococcus epidermidis*) were cultivated in the presence of human sebum fatty acids before transferring them to a lipid-free RPMI medium for 18 hours. Octadecanoid profiling was then performed in both ethanolic washes and microbial culture supernatants using a chiral Supercritical Fluid Chromatography–Tandem Mass Spectrometry (SFC-MS/MS) method that enables regio- and stereo-isomeric identification and quantification of octadecanoids (3).

The antifungal treatment significantly reduced the abundance of the most predominant dermal fungi, *Malassezia* spp. ($p < 0.0001$), whereas bacterial species were not affected by this treatment.

Out of 218 octadecanoids screened, 96 were quantified and met QC criteria in ethanolic washes, while only 10 were quantified in microbial culture supernatants. Both datasets were dominated by linoleic acid-derived species (54/96 and 6/10 quantified octadecanoids, respectively).

The threo isomers of the linoleic acid-derived 9,10-dihydroxy-octadecenoic acid (9,10-DiHOME) were detected in scalp before treatment, while the corresponding erythro counterparts were absent. After treatment, both threo-9R,10R-DiHOME and threo-9S,10S-DiHOME were <LOQ for 78% and 42% of participants, respectively, with a >2-fold lower concentration when measurable. These 9,10-DiHOME shifts are of interest due to its reported pro-inflammatory properties (2).

Octadecanoid profiling in microbial cultures revealed that only *Malassezia* fungi, not *Cutibacterium* or *Staphylococcus* bacteria, could synthesize this pro-inflammatory octadecanoid, thereby confirming the interest of antifungal scalp treatment. Notably, *Malassezia restricta* synthesized the 9S,10S isomer with 100% stereoselectivity, while *Malassezia globosa* also formed the 9R,10R isomer (22%).

This study shows that alterations of octadecanoid levels can reflect scalp surface dysbiosis.

Protein source shapes the gut microbiome and metabolome in growing rats under moderate protein restriction

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The “great food transition” as reflected in the planetary health diet, describes a global shift toward more plant-based diets to improve human health and reduce environmental impact. This shift will likely undertake a transformation in both protein sources and protein intake. Proteins from animal and plant sources differ in amino acid composition and bioavailability, with substantial variation both within and between sources. These differences have been shown to influence gut microbiome composition and downstream microbial metabolism, including bile acid composition with implications for gut health (1,2). However, the amounts and types of metabolites generated in humans following ingestion of different dietary protein sources remain poorly characterized. Metabolomics offers a comprehensive approach to characterize the levels of endogenous metabolites involved in key metabolic pathways and is a well-established technique for probing diet-induced changes and microbiome-metabolome interactions. The aim of the present study was to investigate the effects of dairy and plant-based protein sources on endogenous metabolic and gut microbial-derived responses including bile acids. For this purpose, 45 young male rats were fed one of three moderately low-protein (10%) diets based on either 100% milk protein (n=15), 100% pea protein (n=15) or a 50/50% mixed diet (n=15) for an intervention period of 8 weeks. Metabolomic approaches including ¹H NMR spectroscopic analysis of serum and intestinal content samples combined with targeted LC-MS for the analysis of bile acids were employed. Additionally, microbiota composition was analyzed by 16S rRNA sequencing on intestinal content samples. Multivariate models of preliminary data revealed a clear separation between dietary groups in their serum metabolome. Several significant metabolites reflected differences in the dietary protein composition (methionine, valine and tyrosine), while others showed differences not directly related to diet content (creatine, histidine and leucine). Findings indicated protein-derived effects on arginine-driven metabolic pathways. Ongoing analyses, including targeted bile acid profiling, will further characterize how protein source shapes the gut microbiome as well as systemic and intestinal metabolic profiles. Identifying metabolite patterns associated with different types of protein may provide insight into how diets can be optimized to support metabolic health while moving toward more plant-based dietary patterns.

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Integrating Untargeted Metabolomics and Bioactivity Screening to Explore of Cameroonian bacteria for Antimicrobials Leads Discovery

Rufin Marie Toghueo Kouipou, Aninda Mazumdar, Tommaso Stefani, Zdenek Kamenik

The rising tide of antimicrobial resistance (AMR) necessitates the discovery of novel chemical scaffolds to develop the next generation of medicines. While actinomycetes remain the premier source of antibiotics, traditional bioprospecting is often hampered by the frequent rediscovery of known compounds. However, recent advances in metabolomic and genomic technologies offer a promising path to revisit these microbial resources and uncover untapped chemical diversity. We conducted an ecological survey across underexplored Cameroonian diverse environments, including forests, mountains, rivers, landfills, ant and termite nests, zoological parks, swamps, and ponds, yielding over 200 diverse bacterial isolates belonging to over 20 genera. Utilizing an OSMAC approach across eight media, over 700 crude extracts were generated from 90 selected isolates. These crude extracts were subjected to LC-MS/MS untargeted metabolomics and screened against a panel of five WHO-priority multi-resistant pathogens (*Staphylococcus aureus* N315, *Enterococcus faecium* V583, *Acinetobacter baumannii* Z13, *Klebsiella pneumoniae* E1267 and *Pseudomonas aeruginosa* K109). Data were processed via MZmine 4.8.0 and dereplicated against GNPS2 and our in-house library of 10,000+ compounds. By correlating bioactivity with the global metabolomics dataset, we successfully distilled an initial pool of 40,000 features down to 195 bioactive leads. Extensive dereplication annotated 39 features effectively isolating 161 features as high-priority candidates for novel antibiotic discovery. Large-scale fermentation and targeted isolation of these leads are currently ongoing. By integrating ecological sampling with a robust prioritization workflow, we aim to expand the curated repository of Cameroonian microbial metabolites and provide new weapons in the fight against AMR.

Keywords: Antimicrobial Resistance, Untargeted Metabolomics, Bioactivity-Correlation, Natural Products Discovery, Cameroon Biodiversity

Chemical Metabolomics – New Chemical Biology Tools to Explore Microbiome Metabolism

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Metabolites produced by the gut microbiome play a crucial and diverse role on host physiology, which are detectable in a wide range of biological samples including feces, plasma, urine, and cerebrospinal fluid. Microbiota dysbiosis has been associated with the development of diseases, however, the metabolic link has yet to be detected. The detailed and targeted analysis of these metabolites is important for the discovery of biomarkers and unknown bioactive molecules.¹

Mass spectrometric metabolomics is the method of choice for identification and quantification of these metabolites. Advanced methods at the interface of chemistry and biology coupled with metabolomics analysis are required but still limited. We have therefore developed a unique and multifunctional chemoselective probe with synthetic ¹³C/¹²C isotopically labelled analogues that allows for comparative and quantitative analysis of metabolites in human samples at low concentrations.² We have termed this unique method quantitative Quantitative Sensitive CHEmoselective MetAbolomics (quant-SCHEMA). Coupled to magnetic beads, this method allows the straightforward chemoselective extraction of metabolites from human samples.^[2-6] This isolation procedure of specific classes of metabolites from sample matrices led to significantly increased mass spectrometric sensitivity by sixth orders of magnitude and facilitates the detection of metabolites at femtomole quantities.

The chemoselective probe was applied for analysis of amine- or carbonyl-containing metabolites in human fecal, urine and plasma samples. We have discovered several metabolites previously unreported and performed metabolic profiling in these sample types and confirmation of the presence of medically relevant gut microbiota-derived metabolites. Furthermore, we applied this methodology in dietary intervention study with 156 samples for nutritional biomarker discovery.^[7] We have successfully found four potential food biomarkers, which has not been reported before. The success of this large-scale application of this methodology results in further validation of this method and encourages the general use of this method in metabolomics studies.

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Advancing Gut Microbiome Metabolomics: A Derivatization-Based Approach

Anna Michl, Tommaso Stefani, Ondřej Hřebíček, Martin Schwarzer, Zdeněk Kameník

Microbiota-associated metabolites play a crucial role in host physiology and disease by mediating host-microbiota communication, providing essential nutrients, and regulating metabolism and immune functions. Understanding these interactions requires a comprehensive chemical analysis of the metabolites. However, their low abundance, diverse physicochemical properties, and analysis in complex matrices present significant challenges, leaving us with no universal method to analyze them all. Chemical derivatization is a powerful approach, particularly for polar metabolites. By modifying the analyte structure, it decreases their polarity and makes them compatible with conventional reverse-phase liquid chromatography.

3-nitrophenylhydrazine (3NPH) is a widely used derivatization agent, which was previously employed in studies targeting well-known microbial metabolites such as short chain fatty acids (SCFA) and other primary metabolites. However, the potential of its applicability can be extended and go beyond to larger datasets, covering the majority of polar primary metabolites as well as known products of microbial metabolism.

Here, we present a large-scale analysis of 642 gut microbiome-related chemical standards, derivatized using 3NPH. Standards were systematically classified based on structural similarities, biological relevance, and metabolic pathway associations using a Python-based automated categorization pipeline and public metabolomic databases. For streamlined identification, the recently introduced in-silico Derivatization Tool in MetaboScape by Bruker was employed for automated targeted search. Using this method, we were able to analyze 368 standard compounds (57 %), which were found in non-derivatized form (60 compounds), derivatized form (250 compounds), or as unspecified artefact (58 compounds).

In parallel, we developed an additional workflow using SIRIUS software by generating a database of in-silico derivatized SMILES structures and applying it to our dataset. Moreover, we generated a comprehensive SMILES library of derivatized structures from 5,000+ microbial metabolites curated from MiMeDB. With this, we are addressing a lack of annotation sources for derivatized metabolites.

To validate the applicability of this strategy, we utilized the spectral library of derivatized standards for both untargeted and targeted analysis of microbiota-associated metabolites in 3NPH-derivatized stool samples from mice with different statuses of microbial colonization, including germ-free models. Following the filtering of derivatized features from the dataset, our workflow revealed a clear separation of animal groups in PCA. Our results demonstrate the utility of this approach in providing valuable insights into host-microbiota metabolic interactions.

Gut Microbiota Depletion Unmasks a Metabolomic Signature of the Gut-Liver Axis in Stress-Induced Visceral Hypersensitivity: Insights from a Pseudo-Germ-Free IBS-D Model

Nazila Dardmeh, Ali A. Moazzami, Masoud Yavarmanesh, Hamid Noorbakhsh

It is becoming apparent that the gut-liver axis is a key contributor to the development of stress-related gastrointestinal dysfunction, yet the extent to which an intact gut microbiota is required for this crosstalk remains unclear. To further investigate this microbiota-dependent interaction the present study employed a pseudo-germ-free (PGF) rat model, generated through antibiotic-induced depletion of gut microbiota, to examine how chronic psychological stress and probiotic supplementation influence visceral sensitivity and hepatic metabolism. Following antibiotic-induced establishment of the PGF model, rats underwent a 10-day water-avoidance stress to induce an irritable bowel syndrome with diarrhea (IBS-D)-like phenotype (n=20), while a sham-stress group served as a control (n=5). After stress induction, animals received either vehicle or one of three probiotic regimens, *Bifidobacterium animalis* subsp. *lactis* BB-12 (P1), *Lactiplantibacillus plantarum* ATCC 14917 (P2) or their combination (P1P2) for 28 days. Visceral hypersensitivity (VH) was assessed by abdominal withdrawal reflex (AWR) scoring at graded colorectal distension pressures and hepatic metabolic profiles were characterized using proton nuclear magnetic resonance spectroscopy (¹H-NMR) based metabolomics. Stress exposure markedly increased VH, evidenced by elevated AWR scores and a reduced pain threshold compared with controls ($P < 0.05$). Coincident with this behavior change, there was a significant disturbance of liver metabolism: elevated levels of branched-chain and aromatic amino acids, one-carbon metabolism intermediates, primary conjugated bile acids, choline-related metabolites, and markers of oxidative and osmotic stress were identified. It indicates an increase in catabolic processes and liver insufficiency in meeting its functions for normal energy production and detoxification, at the confluence of microbiota reduction and stress over time. Remarkably, intervention by probiotics, particularly P1 and P1P2, resulted in a substantial decrease in VH and altered hepatic metabolic profiles to resemble controls, suggesting a partial metabolic homeostasis restoration ($P < 0.05$). The current study underscores the importance of the gut microbiota in the maintenance of hepatic metabolic homeostasis and encourages the development of probiotics as potential microbiota-targeted therapies against IBS-D associated visceral and metabolic dysfunction.

Untargeted metabolomics reveals microbial disruption, host tissue damage, and route-dependent vaccine protection during *Histomonas meleagridis* infection

Anh Vu Nguyen, Topi Meuronen, Atte Lihtamo, Soile Turunen, Kati Hanhineva, Olli Kärkkäinen

Histomonosis, caused by the protozoan *Histomonas meleagridis*, is a severe caecal disease of poultry. Histomonosis causes animal suffering and large economical losses, and there is no licensed drug currently available. Although caecal pathology and microbiota disruption have been described, the local metabolic consequences of infection and vaccination remain poorly understood. Here, we used untargeted LC-MS metabolomics to profile caecal tissue and luminal digesta for the first time in a controlled vaccination-challenge chicken trial (n=100 in total, 25 birds/group) comprising uninfected negative controls, and infected chickens with cloacal vaccination, oral vaccination, or unvaccinated positive controls, sampled across five post-challenge timepoints. The results show that *H. meleagridis* infection caused extensive, time-dependent disruption of the caecal metabolome, with the strongest changes during 7-14 days post-infection (DPI). In unvaccinated challenged birds, 325 identified metabolites in tissue and 359 in digesta were significantly altered at DPI 14 relative to controls. Infection was associated with broad depletion of microbiota-associated metabolites, including dicarboxylic acids, tryptophan-derived indoles, phytochemical transformation products, bilirubin catabolites, phenolic compounds, vitamin-related metabolites, and secondary bile acids, together with selective accumulation of microbial substrates and fermentative products. In parallel, changes in the host-associated metabolites indicated mucosal inflammation, epithelial injury, mitochondrial and amino acid metabolic disturbance, oxidative stress, and membrane lipid remodeling. Our dataset also indicates that cloacal vaccination provided broader metabolic protection than oral vaccination, particularly in digesta, where 78.3% of infection-disrupted metabolites were protected at DPI 14 compared with 60.7% after oral vaccination. By DPI 21, cloacally vaccinated birds showed partial-to-complete metabolic recovery across most pathway groups, whereas orally vaccinated birds retained broader metabolic disturbance. These findings define histomonosis as a disruption of the caecal host-microbiota metabolic interface and provide a metabolite-level framework for future studies of pathogenesis and vaccine-based protection of host-microbiome interactions.

P41

Metabolic impact of PFAS and environmental factors on breast milk and microbiota of mother-infant dyads

Matej Orešič, Santosh Lamichhane, Eric Triplett, Johnny Ludvigsson, Mikael Knip, Tuulia Hyötyläinen

Early-life exposure to environmental chemicals, particularly per- and polyfluoroalkyl substances (PFAS) and related persistent organic pollutants (POPs), is increasingly recognized as a key determinant of metabolic health. In this talk, I will present findings from longitudinal mother–infant cohort studies integrating metabolomics, lipidomics, and microbiome profiling to elucidate how these exposures shape early-life biology.

Our work demonstrates that prenatal and early postnatal exposure to PFAS and POP mixtures is associated with profound alterations in lipid metabolism, including ceramides, phospholipids, and acylcarnitines, as well as disruptions in bile acid and amino acid pathways. These metabolic changes are closely linked to shifts in gut microbiota composition and microbial-derived metabolites, highlighting a coordinated perturbation of host–microbiome interactions. Notably, breast milk emerges as a central interface in this process, acting both as a route of exposure and as a modulator of infant metabolic and microbial development.

We further show that different classes of environmental pollutants exert partially distinct yet overlapping effects: PCBs exhibit strong and consistent associations with lipid and microbiome alterations, while PFAS display broader but more heterogeneous metabolic signatures. Across studies, bile acid metabolism and gut–liver axis signaling emerge as key pathways linking environmental exposures to systemic metabolic outcomes.

These findings challenge traditional toxicological paradigms by demonstrating that environmentally relevant exposures reprogram metabolic networks early in life through microbiome-mediated mechanisms. A systems-level, multi-omics perspective is therefore essential to understand how the exposome shapes developmental trajectories and long-term disease risk.

Gut Microbiome Signatures for Cancer Risk: a HUNT4 Study

Nicole Quattrini, Claes Ohlsson, Kristian Hveem, May-Britt Tessem, Monica Engstrøm, Matteo Sangermani, Guro F. Giskeødegård

The gut microbiome has been linked to cancer development through persistent low-grade inflammatory pathways. The release of cytokines and metabolites from bacteria such as *Fusobacterium* trigger inflammation over time and is proposed to be linked with tumorigenesis. Much of the available literature identifies significant findings with individuals recently diagnosed with cancer. Additionally, these discoveries have often been made by (i) not using statistical tools developed for microbiome data analysis, thus resulting in a high risk of false positive findings, and (ii) not adjusting for confounding factors.

Our aim was to identify gut microbiome signatures of cancer development 0 to 6 years prior to cancer diagnosis. Based on the HUNT4 study, we used shotgun sequencing data of the gut microbiome, as well as metabolomic and proteomic data of 11 726 individuals, of which 785 developed their first cancer after faecal sampling.

Differences in taxa between individuals later developing cancer and controls were analysed in (i) all cancers grouped together, and (ii) sub-groupings by the top three cancer types in Norway in 2023: colorectal, prostate, and breast cancer. Alpha and beta diversity analysis were performed, followed by differential abundance analysis using ANCOM-BC2, and MaAsLin3, all analyses were run at a species and genus level. Metabolomic and proteomic data from individuals is being generated. The integration of this multi-omics data with the information on the microbiome differences between the incident cases and controls allows for a detailed picture of the mechanisms through which the gut microbiome may interact with tumour formation.

This study is the largest of its kind looking at prospective cancer incidence. Using a two-method consensus approach to reduce the risk of false positive findings, we identified several significant associations between the gut microbiome and tumorigenesis. The *Fusobacterium* species that have been previously associated with colorectal cancer cases was present in such low relative abundance and prevalence in our data to be filtered out prior to analysis. In the cohort specific to breast and prostate cancer incidence 11 species were identified as differentially abundant, while colorectal cancer identified only one species as differentially abundant, *Prevotella* sp900313215. The species-level analysis on un-specific cancer type identified 12 differentially abundant microbes.

Our findings show that the use of the gut microbiome may be a biomarker for cancer development, and ongoing omics analysis of the same cohort will allow us to examine possible causal pathways.

P43

Gastrointestinal pH and Transit Time Are Associated with Circulating Microbial Metabolites Linked to Cardiometabolic Health

Mariyana Savova, Nicola Procházková, Lars O. Dragsted, Giorgia La Barbera, Henrik M. Roager

Systemic concentrations of gut microbiota-derived metabolites generated from aromatic amino acids have been associated with cardiometabolic health and disease. However, the contribution of gastrointestinal physiology to circulating levels of these metabolites remains poorly understood. In this 9-day observational study, 61 healthy adults underwent assessment of bowel habits, dietary intake, and colonic fermentation as assessed by breath hydrogen and methane levels. Whole-gut and segmental pH and transit times were additionally measured in a subset of 50 participants using wireless motility capsules. Serum samples collected on days 2 and 9 were analyzed by liquid chromatography-mass spectrometry (LC-MS)-based metabolomics to quantify gut microbiota-derived metabolites originating from tryptophan, phenylalanine, and tyrosine metabolism. Higher serum concentrations of the proteolytic metabolites: indoxyl sulfate, phenylacetylglutamine (PAGln), p-cresol sulfate, and p-cresol glucuronide were associated with longer whole-gut transit time. In addition, PAGln, p-cresol sulfate, and p-cresol glucuronide showed positive associations with fecal pH. These findings suggest that gastrointestinal transit time and pH may be important physiological factors influencing the underlying the relationship between gut microbial metabolism and cardiometabolic health and disease.

13C flux ratio analysis with FRAPPPE reveals differences in metabolic fluxes between gut Bacteroidota and Escherichia coli

Daniel Torka, Bartosz Jan Bartmanski, Alexander Spiegelhalter, Inés Herrera-Gomez, Mariana Barcenas, Bernhard Drotleff, Michael Zimmermann, Maria Zimmermann-Kogadeeva

Gut bacteria shape the metabolism of their host and play an important role in human health. However, systems biology approaches to study their intracellular metabolic fluxes are largely underdeveloped. 13C metabolic flux analysis (13C-MFA)¹ has risen as the gold standard to model fluxes in central carbon metabolism, but its application has been largely limited to well-characterized species like *Escherichia coli*² or *Bacillus subtilis*³. The challenges of applying 13C-MFA to non-model organisms arise due to the difficulty of their cultivation in defined minimal medium, and due to potentially unknown rewiring of their metabolic networks necessary for flux simulations. To overcome these challenges, we present an experimental and computational workflow to quantify metabolic flux ratios that combines 13C-labeling with a newly developed machine learning-based Flux Ratio Prediction Python PackagE (FRAPPPE). FRAPPPE utilizes hopsy⁴ and FreeFlux⁵ to sample flux distribution and calculate the corresponding mass distribution vectors. Machine learning predictors are trained on simulated training datasets and applied to experimental data to predict flux ratios. Since FRAPPPE simulates different possible flux distribution scenarios and focuses on specific flux ratios, it can account for network uncertainty and identify flux ratios that are predictable even in more complex culture conditions.

We apply FRAPPPE to investigate central carbon metabolism in two prevalent gut Bacteroidota, *Bacteroides uniformis* and *Phocaeicola vulgatus*, in comparison to *Escherichia coli*. FRAPPPE revealed altered tricarboxylic acid cycle bifurcation in Bacteroidota compared to *E. coli* under anaerobic conditions. Further, we used FRAPPPE to investigate co-metabolism of nucleosides and carbohydrates by *B. uniformis* and *P. vulgatus*. We found distinct species-specific patterns in how nucleosides affected growth and were utilized depending on the co-fed compound. We quantified co-metabolism and showed that the tested nucleosides were mainly contributing to anabolic metabolism closely related to the co-fed nucleoside. However, we observe no major shifts in the labeling patterns of metabolites involved in central carbon metabolism, indicating that nucleosides are not utilized extensively for energy production as long as glucose is available. Together, these findings provide insights into central and nucleoside metabolism of two gut Bacteroidota, and showcase FRAPPPE as a generalizable workflow to investigate metabolic fluxes in gut bacteria.

Integrating Spectroscopic and Genotypic Approaches for Alkaloid-Focused Metabolomics in Perennial Ryegrass

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Endophytic fungi contribute to the perennial ryegrass metabolome through the production of bioactive alkaloids that affect plant fitness, forage quality, and livestock health, and may also influence plant metabolic expression. Key metabolites including peramine, ergovaline, epoxy-janthitrem-I, lolitrem-B, and N-formylloline represent an important targeted subset of the ryegrass metabolome. These fungal alkaloids are traditionally characterized using liquid chromatography-mass spectrometry (LC-MS) (1), while complementary genotypic tools such as Kompetitive Allele-Specific PCR (KASP) assays are widely used to verify endophyte status e.g. presence, strain identity. Although effective, these approaches are resource-intensive and restrict throughput in large-scale metabolomics studies.

This research investigates near-infrared spectroscopy (NIRS) as a rapid, non-destructive analytical platform to support alkaloid-focused metabolomics in perennial ryegrass. A large and diverse sample set representing multiple cultivar-endophyte combinations was characterized using LC-MS for alkaloid profiling, KASP assays for endophyte confirmation, and Fourier-transform NIR spectroscopy (Bruker MPA II) for spectral fingerprinting (2). Spectral datasets were processed using established preprocessing strategies and explored using supervised and unsupervised multivariate approaches to assess metabolic variation associated with endophyte presence and host genetic background.

To link spectral variation with underlying metabolite composition, a range of chemometric and data-driven modelling approaches was applied, including partial least squares discriminant analysis (PLS-DA) and artificial-intelligence-based workflows. PLS-DA was the most robust method, showing reliable performance in both the pooled dataset and cultivar-specific models. Using a nested class approach, it distinguished *Epichloë* endophytic strains with >99% accuracy. Classification of endophyte presence/absence reached ~99%. Peramine, ergovaline and N-formylloline were predicted with accuracies in the ~97-98% range, while epoxy-janthitrem-I achieved ~100% accuracy across cultivars, supporting high-throughput NIRS phenotyping alongside LC-MS validation. A metabolomics-oriented perspective was adopted when interpreting chemical heterogeneity and metabolic variation within the spectral datasets. By integrating spectroscopic data with chemical and genotypic information, this study highlights the potential of high-throughput metabolomic phenotyping approaches to complement conventional mass-spectrometry-based pipelines for characterizing alkaloid variation associated with *Epichloë* endophytes and diverse endophyte strains in forage and agricultural systems.

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Beyond Glucose Transport: Off-Target Effects of SGLT2 Inhibitors Reveal Mitochondrial and Transporter Remodeling in Kidney Disease

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Introduction: Sodium–glucose cotransporter 2 inhibitors (SGLT2i), originally developed to lower hyperglycemia, confer robust renal and cardiovascular protection even in non-diabetic individuals. These benefits cannot be fully explained by inhibition of renal glucose reabsorption, suggesting additional, SGLT2-independent mechanisms.

Methods: To identify off-target effects, we performed integrated proteomic and metabolomic profiling using LC–MS/MS in SGLT2 knockout (SGLT2KO) and wild-type mice across four conditions: healthy, diabetes, obesity, and nephropathy. Disease models included Western diet-induced obesity, Akita-driven type 1 diabetes, and aristolochic acid-induced nephropathy. Cross-species validation was achieved by comparing murine data with plasma and urine profiles from individuals with familial renal glucosuria (genetic SGLT2 deficiency) and SGLT2i-treated patients with and without chronic kidney disease. Direct protein–drug interactions were assessed using thermal proteome profiling in kidney tissue, complemented by short-term pharmacological studies in a microfluidic system. Proteomics was performed label-free on an Exploris 480 platform, and targeted metabolomics on a triple-quadrupole LC–MS.

Results: We generated a comprehensive multi-model atlas of SGLT2-dependent and -independent effects. SGLT2KO signatures were conserved between mice and humans, validating translational relevance. Notably, SGLT2i induced substantial off-target remodeling of renal transport processes, with the strongest effects observed in nephropathy. Chronic kidney disease was characterized by pronounced mitochondrial dysfunction, which was partially rescued by SGLT2i independently of SGLT2. Thermal proteome profiling identified direct binding to multiple solute carrier (SLC) transporters and components of the mitochondrial respiratory chain.

Conclusions: SGLT2 inhibitors exert significant SGLT2-independent effects in the kidney, targeting SLC transporters and mitochondrial pathways. Modulation of mitochondrial function may contribute to their cardio-renal protective phenotype. These findings expand the mechanistic framework of SGLT2i and highlight novel targets for therapeutic intervention.

P47

Untargeted metabolomics supports ferroptosis-associated changes induced by costunolide in Jurkat T cells

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Background and Aim

Costunolide is a sesquiterpene lactone, belonging to a major class of bioactive natural products derived from, the root of *Saussurea lappa* used in Traditional Chinese Medicine (TCM) and has been reported to exhibit therapeutic effects against rheumatoid arthritis.¹ Costunolide has been extensively studied for its diverse biological activities, particularly its anti-inflammatory effects, although its mechanism of action remains unclear.¹ In this project, we performed proteomics and metabolomics analyses to investigate the effect of costunolide in Jurkat T cells. Based on proteomics findings, ferroptosis was identified as the most significant upregulated pathway following costunolide treatment. Ferroptosis is a distinct form of regulated cell death characterized by iron-dependent lipid peroxidation and is closely associated with cellular redox metabolism and polyunsaturated fatty acid (PUFA) oxidation.²

Method

Jurkat T cells (4 million) were cultured, stimulated with anti-CD3 and anti-CD28, followed by stimulation with either DMSO, or costunolide at 8 μ M. Cell pellets were collected for proteomics analysis, while the cell culture supernatants were subjected to metabolomics analysis. 200 μ L of the supernatant underwent protein precipitation with methanol, followed by centrifugation, drying, and reconstitution in 50 μ L of 2% methanol. Samples were analyzed using UPLC - QTOF MS and a C18 column. MS/MS analyses were performed in both ESI+ and ESI- for comprehensive metabolite profiling. Raw data were imported to Metaboscape v.2023 for peak detection and retention time alignment. A feature table was generated, followed by multivariate and univariate statistical analyses performed in R (v. 4.5.1) to identify significantly altered metabolites. Metabolite annotation was performed using an in-house spectral library in combination with public MS/MS spectral databases (KEGG, HMDB, LipidMaps, PubChem) based on fragmentation patterns and accurate mass.

Results

The metabolomic method demonstrated high reproducibility, as confirmed by quality control samples, with relative standard deviation (RSD) less than 20 %, retention time shift less than 0.02 min and mass error less than 5 ppm. A feature table consisting of 1951 and 3985 features was generated in positive and negative mode, respectively. Metabolites of different compound classes were detected, including amino acids, fatty acids, vitamins, and carbohydrates, leading to the identification of 75 metabolites. Among these, 45 metabolites were found to be significantly different (BH-adjusted $p < 0.05$) between costunolide treatment and vehicle control. Of these, 13 metabolites were identified within ferroptosis-related pathways through pathway analysis and literature. We observed significant (BH-adjusted $p < 0.05$) increased levels of PUFAs, including arachidonic acid, adrenic acid, docosahexaenoic acid, docosapentaenoic acid and linoleic acid after costunolide treatment.

Conclusions

In conclusion, this untargeted metabolomics suggests metabolic changes associated with ferroptosis in costunolide treated Jurkat cells, characterized particularly by increased levels of PUFAs that act as substrates for lipid peroxidation. These findings support the hypothesis of ferroptosis upregulation from proteomic analysis.

Untargeted metabolome - microbiome correlation studies in Brassicaceae plants with alternative network visualization reveal an offset between N- and phenylpropanoid metabolism

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The Brassicaceae plant family contains many crops, nutraceuticals and a models for plant biology. Though their chemistry is relatively well studied, phenomena exist which are still best examined with a completely untargeted approach.

Herein, the results of a multi-year field study are presented. In this work, horseradish roots were sampled for 3 consecutive years from a production site of homogeneous argotechnology with various soil types. Features from soil chemistry, fungal microbiome from soil and plant microbiome as well as untargeted metabolomics were assessed for connections. Metabolomics was conducted using LC-MS. A hierarchical color scheme approach applied on the correlation network enabled identification of large patterns that dominate the dataset.

We show that year-to-year variability in the plant metabolome shows dependence of soil texture: amino acid pathway products including glucosinolates show higher year-to-year variability on more loose soils, while other pathways seem to be more robust. In addition, we found that the soil chemistry and roughly 50% of the soil microbiome correlates with a shift from phenylpropanoid - saccharide metabolism towards nitrogen-containing chemical species including glucosinolates and alkaloids. Also, we showed that the plant chemistry is influenced to a larger extent by the soil fungi incapable of plant colonization. In other words, niche categories, taxonomical groups and functional guilds of fungi showed different link patterns towards groups of plant chemical features.

We therefore conclude that soil and plant microbiome analyses should take into account the niche overlap: separation of soil fungi into colonizer and non-colonizer groups and splitting endosphere fungi into ASVs of soil origin and unknown origin significantly increases the depth of insight into plant microbiome assembly. In addition, hierarchical grouping of annotated features from plant metabolomics and fungi (along origin, functional guild or taxonomy) increases the ability to identify large-scale phenomena of such datasets.

Multimomics of fecal extracts reveals TAK-875-associated microbiome and biological changes in a rat model

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Fasiglifam (TAK-875) is an orally bioavailable, selective GPR40/FFAR1 agonist developed for the treatment of type 2 diabetes by promoting glucose-dependent insulin release. Although early dose-finding studies indicated a reduced risk of hypoglycemia, clinical development was discontinued during Phase III trials because of concerns related to liver toxicity. Previous metabolism studies have shown that most TAK-875 metabolites are eliminated in feces, while increasing evidence highlights bidirectional interactions between drugs and the gut microbiome that may influence drug exposure, efficacy, and safety. Here, we applied an integrated multi-omic strategy to investigate TAK-875-associated microbiome and biological changes in a rat model.

The study included 15 male and 9 female Sprague Dawley rats, initially pair-housed and subsequently singly housed during a 96-hour study period. Animals received fasiglifam intravenously (5 mg/kg) or orally (10 or 50 mg/kg), with corresponding vehicle controls. Fecal samples and biological fluids were collected from predose to 96 hours. Fecal material was extracted for polar metabolites using Folch extraction and for proteins, which were reduced, alkylated, and digested using filter-aided sample preparation.

Metabolite extracts were analyzed using a 5-minute HILIC-UHPLC method coupled to multi-reflecting Q-TOF mass spectrometry. Proteomic datasets were acquired on the same MS platform using capillary LC with data-dependent and data-independent acquisition, including stepping quadrupole acquisition and Enhanced Duty Cycle to improve MS/MS sensitivity and sequence coverage. Protein database searches used curated rat and gut-microbial FASTA databases informed by shotgun metagenomics.

Integrated proteomic and metabolomic analyses revealed coordinated dysregulation across the time course, consistent with TAK-875 pharmacometabodynamics. Notably, increased expression of G proteins including GPR84 and GPR43 suggested altered regulation of medium-chain fatty acid signaling and immune-related short-chain fatty acid pathways. These findings demonstrate the value of fecal multimomics for linking xenobiotic metabolism, microbiome function, and host biological response.

Ketogenic communication in polycystic kidney disease

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is characterized by cyst growth in both kidneys, leading to a decline in kidney function. Recently, it was discovered that a dysregulated metabolism contributes to the pathophysiology of ADPKD. Altering metabolism via a ketogenic intervention could be a new treatment option. During a ketogenic intervention, metabolism changes profoundly. Therefore, the aim of this study was to identify metabolic changes induced by ketogenic interventions and evaluate their therapeutic effects in both human cohorts and animal models of ADPKD. We quantified 156 serum metabolites in ADPKD patients on KD, with 18 remaining consistently altered, including TCA, one-carbon, tryptophan/kynurenine, and urea cycle metabolites. Urine metabolomics showed increased uremic toxins excretion on KD, supported by PKD rodent models, underlying KD's detoxifying effect. TCA metabolites were reduced, and glycolysis-related metabolites increased. Hypotaurine, carnitine, kynurenine, tryptophan, tyrosine, and phenylalanine decreased in both humans and rodents, indicating shared amino acid responses to ketosis. Given the metabolic similarities between human and rodent data, we performed kidney cortex proteomics in a rodent PKD model. We found that Slc5a8 (ketone transporter), Bhmt, Csad (both one-carbon metabolism), Vnn1 (CoA metabolism), Glut1, Aco1 (both TCA), and Kmo (kynurenine metabolism) increased in PKD animals compared to WT, showing increased expression on KD, indicating potential metabolic pathway normalization. With ELISA, we confirmed that these changes also significantly reduced oxidative stress, as evidenced by normalization of the reduced-to-oxidized glutathione ratio in kidney cortex tissue. An integrated proteome-metabolome data from all models suggested a sulfuric sink in PKD metabolism, which is targeted by ketosis. However, the metabolomics data of KSD in healthy and ADPKD patients revealed that ketogenic reviving is exclusively driven by KD, but not by ketone bodies supplementation. We found that taurine-bile acid conjugation occurs on KD, potentially affecting cyst growth due to taurine depletion. Therefore, we tested mice supplemented with taurine and cysteamine as a precursor of hypotaurine. That led to increased cystic index (CI) in mice on cysteamine diet compared to the control, however, without changes in kidney weight to body weight. Moltomics data revealed that the ketogenic diet induces beneficial metabolic reprogramming in ADPKD by restoring oxidative metabolism, suggesting KD as a therapeutic strategy and emphasizing dietary intervention over supplementation.

P51

MARC2 deficiency rewires hepatic lipid metabolism and protects against GAN diet-induced liver injury: a sex-resolved multi-omics study

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Mitochondrial amidoxime-reducing components 1 and 2 (MARC1 and MARC2) have been genetically and experimentally linked to hepatic lipid metabolism, yet their distinct roles in the response to chronic nutrient excess remain incompletely understood. We investigated how deletion of *Mtarc1* or *Mtarc2* reshapes the hepatic metabolic response to a 24-week Gubra-Amylin NASH (GAN) diet.

Male and female wild-type, MARC1 knockout, and MARC2 knockout mice were maintained on normal or GAN diet. Liver tissue was profiled using untargeted LC-MS lipidomics and polar metabolomics, ¹H NMR spectroscopy, RNA sequencing, targeted biochemical measurements, and histopathology. Factorial statistical models were used to resolve diet, genotype, sex, and interaction effects, followed by lipid-class and pathway-level analyses.

GAN feeding induced a strongly sex-dependent phenotype, with wild-type males showing the greatest macrovesicular steatosis, lobular inflammation, hepatocyte ballooning, and early fibrosis. MARC2 deficiency produced the clearest protective phenotype. Under GAN challenge, MARC2 knockout males shifted toward substantially lower steatosis grades and showed reduced hepatocellular injury and a milder inflammatory phenotype. Females were less severely challenged by GAN overall, but MARC2-dependent metabolic remodeling remained detectable. MARC1 deletion did not reproduce the marked histological protection of MARC2 loss and instead generated a distinct metabolic response.

Lipidomics revealed extensive genotype-specific remodeling rather than a uniform reduction in hepatic lipids. MARC2-deficient livers were characterized by altered neutral-lipid and phospholipid composition, enrichment of selected very-long-chain diacyl phosphatidylcholines and triacylglycerols, reduced ether-linked phospholipids, and remodeling of acylcarnitine profiles. Complementary metabolomics and biochemical data showed changes in bile-acid and taurine-related metabolism, while NMR independently supported broad differences in hepatic lipid abundance. Transcriptomic pathway analysis converged with the metabolic phenotype, indicating attenuation of GAN-responsive programs associated with lipid accumulation, inflammation, cellular stress, and extracellular-matrix remodeling.

Together, these data reveal a striking divergence between MARC1 and MARC2 and identify MARC2 as a major regulator of hepatic adaptation to dietary metabolic stress. Integrating lipidomics, metabolomics, NMR, transcriptomics, and histopathology shows that MARC2 deficiency reorganizes hepatic metabolism while strongly limiting progression toward steatohepatitis.

P52

Integrative Metabolomic and Microbiome Profiling Identifies Candidate Biomarkers and Pathological Mechanisms Associated with Coronary Artery Disease

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Coronary artery disease (CAD) is a leading cause of mortality worldwide. CAD is caused by the formation of atherosclerotic plaque in the lumen of coronary arteries. Stenosis refers to a significant narrowing of the arterial lumen by advanced atherosclerotic plaque formation, resulting in impaired blood flow and reduced oxygen supply to the myocardium. Sclerosis refers to atherosclerotic changes and plaque deposits within the vessel wall, leading to thickening and hardening of the arteries, but not necessarily causing a flow-limiting narrowing. Stenosis and sclerosis are often mixed up in diagnosis, particularly at early stages. Thus, identifying distinct biomarkers associated with the development of coronary stenosis and sclerosis is crucial for guiding precise and early therapeutic strategies. A total of 48 plasma samples from patients with stenosis (N = 23), sclerosis (N=12) and disease controls (N=13) were analyzed. Metabolites and lipids were targeted and quantified using the Quant 1000 kit from biocrates. Next generation sequencing was used to generate microbiome data by mapping microbial reads using an in-house built pipeline to obtain microbe abundances.

Several models were applied to integrate the two omics and explain group variance: Multi-Omics Factor Analysis (MOFA) infers latent factors that explain variance, Group Aggregation via UMAP Data Integration (GAUDI) identifies clusters through non-linear dimensionality reduction, canonical correlation analysis (CCA) maximizes correlation between the omics and was used to evaluate the associations between single metabolites and microbiome strains. Our analysis found three multi-omics clusters: one dominated by stenosis, one dominated by disease controls and one including patients from all the groups. The multi-omic interaction network showed two modules: one made of triglycerides (TG) with associations to Capnocytophaga and Cryptococcus, and another associating phospholipids with Corynebacterium, Citrobacter and Clostridium, among others. Combining analyses with different integration strategies, distinct profiles for both microbiome and metabolome were identified for all groups

P53

Metabolomics and Proteomics Approaches to Identify New Marker of Nitrofurazone in Pig Hepatocytes Cell

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Nitrofurazone (NFZ) is a synthetic nitrofuran antimicrobial that has been widely used since 1940s as feed additive for preventive and curative veterinary medicine in livestock and fish farming. Due to its potential carcinogenic and mutagenic effects, its use in food-producing animals has been banned in many countries. Nitrofurazone is rapidly metabolized to give a protein-bound metabolite, the semicarbazide (SEM), which is currently used as marker residue for nitrofurazone abuse. However, SEM lacks specificity, leading to a less-conclusive marker when generated from other sources, including food processing and packaging materials. The present study aims at investigating by in vitro systems (suspensions, 2D, and 3D cultures) the metabolism of NFZ in porcine primary hepatocytes cells, using LC-Q-Orbitrap high-resolution mass spectrometry to identify new markers through metabolomic approach. A pilot study evaluates cytotoxicity and metabolic responses across a range of NFZ concentrations to define optimal experimental conditions. In parallel, proteomic approach using nano-LC-TIMS-TOF is also applied to investigate the formation of NFZ protein-bound metabolites capable of altering protein structure/conformation and thus leading to potential biomarker for nitrofurazone abuse. Finally, the identified metabolites could serve as new alternative markers for regulatory applications and solve the critical gap for the detection of illicit nitrofurazone use in food-producing animals.

Multi-omics profiling of the enteral/parenteral nutrition transition in very preterm infants reveals coordinated monocyte, metabolome, and cytokine signatures

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Background: In very preterm infants, parenteral nutrition (PN) bridges the period before full enteral nutrition (EN) with human milk is tolerated. A higher PN share has been linked to several preterm morbidities, but the molecular changes that accompany the EN/PN transition remain incompletely described. **Methods.** Thirty-two infants born before 30 weeks of gestation were profiled at postnatal days 7 to 11 using CD14⁺ monocyte RNA sequencing, untargeted plasma metabolomics (Metabolon HD4, 1,442 features), plasma proteomics (819 proteins after filtering), and Bio-Plex cytokine profiling (77 analytes). The cumulative EN/PN ratio at sampling spanned 0.04 to 1.0 (median 0.81). DESeq2 (RNA-seq) and limma (other platforms) modelled the ratio with gestational age as a covariate; pre-ranked enrichment used KEGG, Reactome, GO, and Metabolon ontologies, with Benjamini-Hochberg FDR < 0.05.

Results: After GA adjustment, 229 of 23,310 monocyte genes were associated with the EN/PN ratio at FDR < 0.05; pathway-level GSEA returned 58 KEGG, 178 GO BP, and 248 Reactome enriched terms covering cell cycle, oxidative phosphorylation, MHC class II antigen presentation, and IL-17/NF- κ B signalling. Cell-cycle markers, CD163, VSIG4, and IL10 declined while a differentiation cluster (NR4A1, ALDH1A1, JUP, ITGA4) increased. Of 819 plasma proteins, none reached FDR < 0.05. Of 1,442 plasma metabolites, 218 were associated with the EN/PN ratio at FDR < 0.05. Plasma free fatty-acid profiles mirrored substrate compositions: EPA, DHA, and milk-distinctive medium-chain saturates (caprate, laurate, myristate) rose, while linoleate (Linoleic-dominant) declined. Free tyrosine and the downstream catabolites 4-hydroxyphenylacetate and 3,4-dihydroxyphenylacetate sulfate rose as enteral feeding replaced Vaminolac's solubility-constrained tyrosine dose. Microbial diketopiperazines (cyclo(gly-pro), cyclo(ala-pro), cyclo(leu-pro)) and PEPT1-transported dipeptides rose while 14 conjugated bile acids declined. Membrane lipid classes characteristic of the milk fat globule membrane (sphingomyelins, phosphatidylcholines, plasmalogens, lyso-phosphatidylcholines) were enriched at higher EN/PN. Of 77 cytokines, five reached FDR < 0.05 (soluble CD163, IL-2R α , IFN- β , IL-35, IL-6), all declining; soluble CD163 in plasma tracked its monocyte mRNA, providing cross-platform validation of the monocyte-state shift.

Conclusion: Increasing enteral nutrition at postnatal day 7 to 11 is independently associated with a coordinated transition: monocytes shift from an emergency-myelopoietic, scavenging phenotype toward antigen presentation; gut-microbial metabolites appear in plasma; PN-associated bile-acid accumulation resolves; and plasma lipid composition tracks substrates delivered by enteral milk.

Elucidating the metabolic characteristics in the developing human liver through genome-scale metabolic modelling

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Per- and polyfluoroalkyl substances (PFAS) are endocrine disrupting chemicals that continue to be used in many industrial products. PFAS exposure alters metabolite levels including bile acids, increasing susceptibility to the onset and severity of various non-communicable diseases. Biomonitoring studies reveal that PFAS are detected in almost all humans, including fetuses, raising serious concerns for long-term health. Recently, we reported that PFAS accumulate in the fetal human liver and are associated with disturbances in lipid metabolism, including bile acid metabolism (1). Therefore, identification of specific metabolic pathway perturbations caused by PFAS exposure in the human fetus is needed. Here, we investigated metabolic pathways of the human fetal liver using omics data and a constraint-based modelling approach. We integrated liver proteomics data from 56 human fetuses (gestational ages between 12 and 19 weeks) with a comprehensive genome-scale metabolic model, Human1 (2), to predict metabolic flux profiles. Our analysis revealed metabolic reprogramming in the developing liver, as well as its dysregulation by PFAS exposure. We observed that replication-related pathways are more active at early gestational age (12–14 weeks). Furthermore, we identified several PFAS-associated metabolic signatures, specifically highlighting alterations in lipid, amino acid, nucleotide, pyruvate, glycan and vitamin metabolism. Interestingly, model predictions revealed that PFAS negatively impact steroid metabolism, including estrogen and androgen metabolism. To confirm the impact of PFAS exposure on the fetal liver steroidome, we analysed liver samples from an independent cohort of 84 fetuses (gestational ages between 11 and 20 weeks) by both targeted (steroids) and untargeted mass spectrometry-based metabolomics analyses. Androstenedione was inversely ($\beta = -0.34$, $p < 0.001$) and 17β -estradiol ($\beta = 0.21$, $p = 0.042$) was positively associated with PFAS. Moreover, ratios of substrate steroids over product steroids were inversely associated with PFAS, indicating that PFAS disrupt steroidogenic enzyme activity. In conclusion, PFAS exposure is associated with disturbed liver development, including altered production of important steroids.

Lipidomic and metabolic characterization of ocular retinoblastoma model and investigation of tumor targeting properties of PL3 peptide using LC-MS and MALDI-MSI

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Retinoblastoma is the most common intraocular tumor among children under 5 years of age. To salvage the eye, early intervention is necessary before the disease progresses to a severe stage. Current treatments include systemic, intraocular, and intra-arterial chemotherapy. Even though they are often effective, these treatments are known to have systemic or possibly vision-affecting side effects. Therefore, a more tumor-targeted approach with fewer side effects is under investigation. Loss of functional retinoblastoma protein is the most common genetic alteration in retinoblastoma, and its knockout is linked to altered lipid metabolism. [1,2].

This study aims to characterize the lipidomic and metabolic profiles of retinoblastoma using human Y79 retinoblastoma cells and to compare them with those of healthy retina. In addition, the study investigates the intracellular effects and cancer-targeting properties of the PL3 peptide as a promising new drug delivery strategy for retinoblastoma. Methods in this study include mass spectrometric imaging (MSI) of human Y79 retinoblastoma, ARPE19 retinal pigment epithelial cells and rabbit ocular tissues. UHPLC-HRMS lipidomics and metabolomics analyses are used to putatively identify the molecular features detected by MSI. Light microscopy is used to acquire scanned histological images of MSI-analyzed slides, enabling co-registration of the MALDI images with hematoxylin- and eosin-stained biological structures.

Preliminary MALDI imaging results from co-cultured Y79 and ARPE19 cells suggest that these two cell lines have distinct lipid profiles and that spatial lipidomics could be applied to identifying tumor-associated molecular profiles. Region-specific molecular features detected by MALDI-MSI were compared with lipids and metabolites identified by UHPLC-HRMS.

UHPLC-HRMS lipidomics and metabolomics analyses were done for naïve cultured Y79 cells and for Y79 cells treated with either PL3 peptide or control peptide. In addition, lipidomic and metabolic profiles of healthy rabbit retina were analyzed.

To further interpret these findings, the spatial localization of the identified features was investigated in rabbit eye tissue sections using MSI. Features observed in cell cultures were compared with their distribution in rabbit retina to assess their natural localization in healthy tissue.

Overall, these results will provide a detailed characterization of lipidomic and metabolic profiles in retinoblastoma and the healthy retina and demonstrate the applicability of MSI for identifying tumor-associated molecular features in a disease model and for studying targeted drug delivery strategies for retinoblastoma.

P57

High-throughput Single-cell Multi-OMICS Using Standard-flow LC and a Multi-reflecting TOF MS

Otto Savolainen, Jayesh Kattla, Matthew Daly, Lee Gethings, Richard Lock, David Heywood

Single-cell lipidomics and metabolomics are increasingly important for uncovering cell-to-cell biochemical heterogeneity that is often masked in bulk analyses. However, the extremely low analyte abundance in individual cells requires sensitive, robust and scalable analytical workflows. Here, HT29 and CaCO2 gastrointestinal and cancer research cell lines were used to demonstrate a unified LC-MS strategy for high-throughput discovery profiling. Single cells were isolated using the IsoPick microfluidic platform and deposited directly into LC vials. Lipids were extracted with ice-cold isopropanol containing deuterated internal standards, while polar metabolites were extracted with 0.1% formic acid using a validated method. Extracts were analysed on a U(H)PLC system equipped with a 2.1 × 100 mm phenyl-hexyl column operated at 0.4 mL/min and 70 °C, coupled to a multi-reflecting TOF mass spectrometer running DIA and DDA modes with enhanced duty cycle (EDC). Processed datasets produced more than 200 lipid identifications and over 150 polar metabolites per single cell (n = 5), spanning amino acids, organic acids and nucleotides. High-resolution MS1 and MS2 data, boosted by EDC for approximately 10-fold higher MS2 signal, improved confidence in structural elucidation. The phenyl-hexyl chemistry eliminated isopropanol from the mobile phase, reducing background signal and enhancing signal-to-noise. This streamlined LC-MS configuration enables comprehensive single-cell lipidomics and metabolomics without hardware changes, providing an efficient and scalable platform for multi-OMIC discovery studies.

Spatial characterization of prostate cancer reveals antigen-presenting cancer-associated fibroblast- rich niches linked to disease aggressiveness

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The tumor microenvironment (TME) of prostate cancer (PCa) is a complex ecosystem, yet its spatial heterogeneity across disease states remains poorly characterized. We analyzed 32 fresh-frozen tissue sections from 8 treatment-naïve PCa patients, including 5 patients with biochemical recurrence (PSA > 0.2 ng/mL; aggressive) and 3 patients who remained relapse-free for over 9 years following radical prostatectomy (non-aggressive), using spatial transcriptomics (ST) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI). We integrated ST and MALDI-MSI data with detailed histopathological annotations to spatially characterize the PCa TME and identify functionally organized niches associated with clinical aggressiveness.

Spatially aware unsupervised clustering of ST spots identified niches distributed across tumor, tumor-adjacent, and distant-normal compartments. Functional enrichment analysis further grouped these niches into functionally related types. Notably, one niche type was enriched for antigen-presenting cancer-associated fibroblasts (apCAFs) and was consistently observed in tissues from patients with aggressive PCa. The apCAF-rich niche showed significantly closer proximity to lymphocyte-rich regions than other CAF-containing niches ($p=0.017$, Wilcoxon test) and increased T-cell exhaustion signatures. Correspondingly, MALDI MSI revealed elevated stromal glucose, ATP, and ADP levels ($p<0.01$) within apCAF-rich niches, suggestive of increased energetic turnover and purinergic signaling, both of which have been associated with T-cell exhaustion-related metabolic remodeling [1].

Altogether, through an unsupervised integrated multi-omics framework, we identify a spatially distinct immune-metabolic stromal niche characterized by apCAF enrichment, chronic immune dysfunction, and association with aggressive PCa, highlighting its potential as a prognostic biomarker and therapeutic target. These findings were further supported across independent cohorts with long-term clinical follow-up (TCGA, $n=486$, $p=0.02$; GSE54460, $n=98$, $p<0.01$; GSE220095, $n=176$, $p<0.04$; log-rank test), as well as in an external spatial transcriptomics cohort (GSE245388).

P59

Integrative Multi-Omics Characterization of Radiomic Phenotypes in Prostate Cancer

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Prostate cancer (PCa) is a highly heterogeneous disease which poses a challenge in diagnosis and treatment of patients, particularly the distinction between non-aggressive and aggressive PCa. Linking molecular tumor characteristics to in vivo magnetic resonance imaging (MRI) could enable the non-invasive molecular characterization of PCa, ultimately leading to a more accurate diagnosis and improved patient care. However, accurately capturing the spatially heterogeneous nature of PCa requires a precise spatial alignment between MRI and molecular data at an intratumoral level. In this work, we developed a novel semi-automated spatial matching pipeline that integrates molecular multi-omics measurements (metabolomics, transcriptomics) and histopathology from fresh-frozen tissue samples with radiomics features from biparametric MRI (T2-weighted imaging, apparent diffusion coefficient maps, and high b-value images). The pipeline spatially localizes tissue sample sites in adjacent whole-slide images, followed by image registration of whole-slide images to their corresponding MRI slices using T2-weighted images as an anatomical reference. To highlight the potential of our approach, we performed an integrative radio multi-omics analysis (N = 3 patients, n = 11 samples) that identified statistically significant associations between molecular PCa characteristics (TCA-cycle, lipid synthesis, cell cycle progression, and changes in extracellular matrix organization) and MRI features (high b-value intensity and T2-weighted texture features). In conclusion, we present a spatial matching pipeline for aligning ex vivo molecular measurements with in vivo MRI and demonstrate its potential to molecularly characterize PCa-related imaging phenotypes.

13C Fluxomics based on trusted LC-MS data

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Multionomics provides a comprehensive overview of a biological system through integration of data such as genomics, proteomics, and metabolomics. Fluxomics as a part of the multionomics field utilizes quantitative data to estimate reaction rates within a biological system providing an opportunity to capture the dynamics of a cellular metabolic network. However, fluxomics is less commonly applied than other multionomics techniques and requires precise and consistent measurements to accurately characterize cellular metabolism.

Fitting methods often use relative abundances of mass isotopomer distributions (MID) for selected metabolites as an input. Despite the advancements in the quantitative omics, achieving consistent measurements and accounting for bias when using different analytical platforms remains challenging. To evaluate the precision of collected information, a bespoke measurement model can be introduced to account for instrument specific data variability and ensure more consistent analysis of metabolic fluxes.

Our poster presents an integrated LC-MS based fluxomics workflow that simultaneously fits the metabolic fluxes and quantifies the LC-MS' uncertainty. We describe the steps of metabolome extraction of HEK293 and Red Blood Cells (RBC), data acquisition, evaluation, and modelling of the metabolic fluxes using a 13C based Metabolic Flux Analysis.

To efficiently capture 13C labelled metabolic profiles, two protocols were applied for HEK and RBC cells. HEK metabolome was extracted from cell pellets using ACN:MeOH:H₂O (2:2:1). RBC metabolome was collected using 0.45 µm PVDF filters that were vacuum-dried and submerged in the same extraction buffer. Collected supernatants were dried and reconstituted in water for further analysis.

Data acquisition was based on two targeted ion-pairing LC-MS/MS methods collecting the isotopologue signals for 21 compounds of central carbon metabolism. Both 1,2 and U-13C tracers were used to acquire steady state and time course fluxomics data. The 13C enrichment fraction was evaluated over time, and isotopologues abundance was transformed into MID measurements.

Information on tracer, MID measurements, and a reaction map were utilized as an input into a differentiable Bayesian 13C MFA network to infer metabolic fluxes. The MFA network was extended with a dual saturation measurement model that accounts for low and high abundance isotopologue measurements. The model uses 12 levels based dilution dataset of a biological HEK sample to estimate the measurement error both per metabolite and isotopologue.

Presented fluxomics workflow allows to collect the data on 13C enriched metabolome of mammalian cells and evaluate metabolic fluxes through a bias aware modelling of LC-MS data tailored to a specific instrument.

The abundance and therapeutic potential of lipid droplets in Multiple Myeloma

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Multiple myeloma (MM) is a heterogeneous haematological malignancy originating from the bone marrow. One of the main challenges facing patients diagnosed with MM, is that despite the recent advances in treatment and disease management, many patients will eventually relapse and develop treatment resistance. Interestingly, in MM, no single genetic alteration has been established as principal pathogenic event highlighting the role of the intertwined dysregulation of several molecular mechanisms, including metabolic reprogramming, in MM development. Notably, malignant plasma cells dynamically alter key metabolic pathways to support their elevated energy demands resulting in disease progression. Previous studies have suggested that cancer cells meet their increased energetic demands needed to maintain a high proliferative rate by enhancing lipid synthesis and storage within LDs. Moreover, elevated LD abundance has been linked to treatment resistance. However, the dependency of LDs in MM has not been investigated yet.

This research aims to evaluate the effect of de novo lipogenesis inhibition on LDs in human and murine MM cells. We identified increased abundance of LDs in MM cells by LD-BTD1 staining. By utilizing RNA-seq data from newly diagnosed MM patients within the MMRF cohort, we demonstrated that key gene transcripts of the de novo lipogenesis pathway (ACLY and SREBP1) were overexpressed in MM compared to healthy individuals and associated with worse prognosis. Interestingly, inhibition of ACLY (BMS-303141) and SREBPs (fatostatin) significantly reduced cell viability and induced apoptosis in human and murine MM cells. Furthermore, combinatorial treatment with bortezomib, the standard-of-care for newly diagnosed MM patients, and BMS-303141/fatostatin significantly increased cell death and apoptosis in human and murine MM cells compared to control and the single treatments. To shed light on the underlying mechanisms promoting these anti-MM effects, we will investigate the downstream impact of the treatments via metabolic and lipidomic profiling, using liquid chromatography coupled with mass spectrometry and by RNA-seq. To investigate the therapeutic potential of the combinatorial treatment in vivo, we will evaluate its effect on tumour growth and mouse survival in the 5T33MM immunocompetent syngeneic murine MM model and in an OPM2 xenograft (CDX) MM model.

Taken together, our preliminary data suggest that MM cells rely on lipid droplets for their survival and proliferation. Moreover, we demonstrated that combination of bortezomib with de novo lipogenesis inhibition enhanced the sensitivity of human and murine MM cells to the standard-of-care suggesting a novel therapeutic avenue for the treatment of MM.

(Sub)cellular dynamics of arginine and lysine demethylation

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Arginine and lysine methylation levels and dynamics are essential for cell homeostasis, and dysregulation thereof may be a key event in numerous diseases and the hallmark of cancers and aging.

At present, and despite the plethora of studies, arginine methylation is among the few post-translational modifications where there is no convincing evidence that an enzyme “erasing” the modification, a so-called demethylase exists. Recently, in cellular studies and using NMR-spectroscopy, our group showed that arginine methylation is dynamic in cells and that the overall dynamics of arginine methylation turnover and demethylation are slow, with half-times of 15 hours to 1 day (1). Lately, arginine demethylase activity has been reported for lysine demethylases, other studies suggest that demethylation may also be achieved through lysosomal or proteasomal degradation (2,3,4) . However, the relevance of these individual pathways leading to turnover of arginine methylation, including the existence of a potential demethylase remain mysterious.

Therefore, we hypothesize that arginine “de”methylation is mediated via proteasomal pathways, lysosomal pathways, and demethylases or a combination thereof. To this end, we aim to first study the impact of selected lysine demethylases on arginine methylation in cells using a combination of NMR spectroscopy-based metabolomics and cell biology techniques.

For now, we found the metabolism of methionine and its inhibitor intervention will significantly affect the state of cells and the level of methylation within cells. There were differences in the effects of different amino acid starvation conditions on cell survival and methylation level. And cancer cells and non-cancerous cells showed different response patterns in methylation regulation. Meanwhile, our preliminary ¹³C tracing results suggest that the metabolic changes of glutamine may vary in the level of involvement in the regulation of methylation processes in different cell lines. These data establishing stable and reproducible methylation baseline data across multiple cell lines and is essential for accurately interpreting the demethylation process and its subsequent recovery dynamics.

In further experiments we will perform more cell experiments to verify the demethylation capability of lysine demethylases and their effects on protein methylation, and we also aim to investigate the relative effects of proteasomal pathways and lysosomal pathways during protein degradation, thereby elucidating the mechanism of intracellular protein turnover.

Matrix Matters: Challenges of Universal Single-Phase Extraction Across Eight Murine Tissues and Serum

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Abstract:

Single-phase extractions using methanol (MeOH) or isopropanol (IPA) are standard protocols in high-throughput metabolomics and lipidomics. Although solvent extraction efficiencies have been widely studied for biofluids such as serum and plasma, protocols are routinely translated to solid tissues under the assumption of transversal behavior. However, systematic evaluations directly comparing solvent performance across diverse tissue matrices remain largely unexplored.

Here, we systematically benchmarked the extraction coverage and matrix-dependent recovery of single-phase MeOH versus IPA across diverse murine tissues and biofluids using multi-platform targeted LC-MS/MS. Serum and eight distinct solid tissues (brain, liver, kidney, heart, lung, spleen, skeletal muscle, and white adipose tissue) were harvested from age-matched male and female mice ($n = 3$ per group), extracted using either single-phase MeOH or IPA protein precipitation, and analyzed across five targeted LC-MS/MS platforms (two polar metabolomic and three lipidomic) covering over 1,500 distinct molecular species.

While IPA yielded superior signal for highly hydrophobic lipids such as fully saturated triacylglycerols (TAGs), the majority of unsaturated TAG species exhibited inconsistent, matrix-dependent recovery profiles between solvents, with MeOH frequently outperforming IPA. As expected, MeOH extracts exhibited broader coverage and greater signal intensity over IPA across all matrices on polar platforms. For instance, compounds like ADP and NAD⁺ were readily detectable in MeOH but were undetectable in IPA. Among polar lipids, matrix-specific divergence was also found. For instance, S1P area in serum was only 50% lower in IPA compared to MeOH, whereas in solid tissues, IPA extraction resulted in a >10-fold area reduction, dropping near or below the detection limit in most cases.

The extraction efficiency of a given solvent is strongly matrix-dependent and cannot be generalized from biofluids to solid tissues, or even across distinct solid tissue matrices. While more readily available serum or plasma matrices allow dedicated dual-solvent extraction workflows, low-input applications (1-2 mg tissue) require an extraction solvent selection based on the specific endpoint.

While this simple binary solvent comparison experiment could be extended to other solvents and ratios, the broader takeaway is that true universal coverage within a single extraction is chemically unfeasible. Analytical compromises are always made and must be considered a priori; in particular, service laboratories and core facilities should transparently communicate these inherent trade-offs so end-users can, if necessary, request extraction strategies tailored to their targets.

P64

Dietary Carbohydrate Quality Exposure and Dementia Risk in UK Biobank Cohort: A mediation Analysis of Plasma Metabolome.

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Background: Emerging research suggests that dietary carbohydrate quality may play a significant role in dementia's risk. However, the role of endogenous plasma metabolome on this association remains to be determined. We investigated the associations between glycemic load exposure and the risk of dementia, and whether plasma metabolites mediate these associations. Methods: A total of 202,201 participants in the UK Biobank (UKB) cohort study were included in the present study. Glycemic load intake was estimated at baseline through validated 24-hour dietary recalls. The metabolomics dataset comprised 167 plasma metabolites. Cox proportional hazards models were employed to estimate the hazard ratios (HR) and 95% confidence intervals (CI). High dimensional and causal mediation analyses were conducted to select potential metabolite mediators and assess their proportion of mediation, respectively. Results: During an average follow-up of 13.2 years, 2,535 overall dementia cases (including 1,121 Alzheimer disease (AD), 456 vascular dementia (VD), and 91 frontotemporal dementia (FTD) cases) were diagnosed. We found significant associations between GL (1-SD increase) and risks of overall dementia (HR = 1.12, 95% CI: 1.06–1.18), AD (HR = 1.14, 95% CI: 1.04–1.24), and VD (HR = 1.19, 95% CI: 1.05–1.36). The associations between GL and dementia were partially explained by glucose, glycoprotein acetyls, total branched-chain amino acids, polyunsaturated fatty acids, phosphatidylcholines, total lipids in VLDL, clinical LDL cholesterol, and the average diameter for VLDL particles, which mediated between 6% and 18% of these associations. Conclusions: Our findings confirm that GL is positively associated with dementia, potentially through the regulation of specific plasma metabolites.

P65

A Comprehensive Lipidomics Workflow Combining Broad Screening, High-Resolution Annotation and Quantitative Validation

Per Jan-Wilhelm Larsson, Anton Ribbenstedt, Otto Savolainen, **Malin Barman**

Background: Lipidomics studies often require a balance between broad lipid coverage, confident molecular annotation and robust quantitative performance. Existing workflows commonly prioritize either comprehensive screening or quantitative accuracy, creating challenges when translating discovery findings into reliable biological measurements.

Methods: We are developing a lipidomics workflow that integrates broad lipid screening, high-resolution mass spectrometric confirmation and targeted quantitative analysis within a unified analytical framework. The workflow combines automated sample preparation, large-scale lipid screening, high-resolution mass spectrometry for annotation support and streamlined data processing. Candidate lipids are selected and refined into study-specific panels that can subsequently be evaluated using quantitative methods.

Results: The workflow enables comprehensive interrogation of multiple lipid classes while supporting improved confidence in lipid annotation and flexible adaptation to different biological sample types. By combining complementary mass spectrometric approaches, the strategy provides a practical route from broad screening to quantitative analysis. Initial evaluations demonstrate the feasibility of integrating screening, annotation and validation steps within a single platform workflow.

Conclusions: This approach provides a scalable framework for lipidomics studies that bridges the gap between comprehensive lipid screening and quantitative validation. The workflow is designed to support a wide range of applications, including biomarker discovery, mechanistic studies and translational research.

The EMBLEM project: Understanding the impact of endocrine-disrupting chemicals and their metabolites on the progression of MASLD

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent chronic liver disease globally, affecting about 30% of adults and costing over €334 billion annually in Europe alone. Among known risk factors, endocrine-disrupting chemicals (EDCs) are of particular concern as they are known to contribute to many chronic diseases. Moreover, EDC metabolism pathways are sparsely documented, leading to an underestimation of both human exposure and toxicity. The EMBLEM project aims to uncover new EDC-related chemical risk factors of the progression of MASLD. The project has three main objectives: (a) Identifying new EDC metabolites through non-targeted analysis in animal and human models, focusing on their impact on MASLD, (b) Characterising EDCs and metabolites in plasma samples from 2,000 MASLD patients using high resolution mass spectrometry data, and (c) Investigating impact of EDC mixtures on MASLD progression by linking exposure data with disease status, using a hypothesis-driven epidemiological approach. With the first objective well on its way, statistical approaches and molecular networking approaches have notably been leveraged to identify new EDC metabolites using data from in-vitro exposure of hepatocytes to PFAS perfluorohexyloctane. Manual structural elucidation of a marker prioritised through a statistical approach led to the discovery of putative biotransformation product perfluorohexyloctanoic acid, which demonstrated distinct biological activity from its parent compound(1). This discovery highlighted the relevance of considering EDC metabolites as separate chemical risk factors for adverse health outcomes. Moreover, the presumed metabolism pathway leading to the biotransformation of perfluorohexyloctane may be applicable for other semi-fluorinated PFAS. Combined with the distinctive MS2 signature of this novel metabolite, featuring sequential and recurring HF group eliminations, this provides a strong opportunity to generalise these findings, attempt detection of predicted metabolites of other semi-fluorinated PFAS in human samples, and investigate their role in MASLD progression.

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Uncovering the Hidden Chemical Diversity of *Alternaria alternata* via Dual-Polarity Molecular Networking Approach

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The fungal genus *Alternaria* is known for producing a vast array of secondary metabolites, including phytotoxins and mycotoxins that pose severe health and agricultural risks¹. Fungi actively biotransform these metabolites into structural variants to facilitate transport, evade host immune defenses, and detoxify their own chemical arsenal. Because these "masked mycotoxins" possess altered masses and low relative abundance, they are notoriously difficult to detect using traditional targeted analytical methods, creating a blind spot in toxicological risk assessments². While untargeted liquid chromatography mass-spectrometry (LC-MS) combined with molecular networking (MN) offers a highly promising solution, data interpretation and annotation remain major bottlenecks in novel natural product discovery.

To deeply explore the *Alternaria alternata* metabolome, we analyzed extracts from 30 different isolates using HPLC-HRMS/MS. To enhance compound discoveries, we implemented a refined computational MN strategy. Specifically, we generated a neutral molecular network that merges denoised fragmentation data from both positive and negative ionization polarities.

The integration of dual-polarity pseudo-spectra improved the spectral informativity and connection reliability within the MN. This robust annotation propagation enabled a faster and more confident characterization of unknown nodes, leading to the discovery of several previously unrecorded biomodifications of known phytotoxins. Key molecular discoveries include novel acyl-variations of tenuazonic acid, distinct hydration and demethylation derivatives of tentoxin, and cysteine conjugates of AM-toxins, which provide evidence of possible detoxification interactions. Furthermore, the improved network topology revealed a completely novel, diversified cluster of pipercolic acid-valine derivatives undergoing extensive glycosylation and peptide extension, suggesting this compound class acts as a central metabolic scaffold for the fungus.

By applying an advanced molecular networking approach, we successfully mapped the hidden metabolic plasticity of *Alternaria alternata*. These findings provide a much more comprehensive understanding of fungal chemical diversity, improving the knowledge about biomodifications that should be considered for accurate toxicological risk assessments.

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Towards an Automated Platform for Single-Cell Metabolomics

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Mass spectrometry-based single-cell omics can reveal cellular heterogeneity at the metabolite level. Here, we present an automated pneumatically assisted nanospray desorption electrospray (PA nano-DESI) platform for single-cell metabolomics. Automated stage movement reduces user dependency, while parallel acquisition of high-resolution Orbitrap full scans with simultaneous ion trap MS/MS improves annotation confidence by resolving isobars and isomers.

Data were acquired on an Orbitrap IQ-X Tribrid mass spectrometer in positive mode (m/z 100–1000). Orbitrap full scans were recorded at 240K resolution, while multiple MS/MS spectra were acquired in parallel in the ion trap. Individual INS-1 cells were dispensed into a 13×7 microwell substrate using the CellenONE system. Automated PA nano-DESI sampling was controlled via programmed linear stages, and data processing was performed using the in-house-developed tool CellMate.¹

Microwell substrates enabled automated sampling of up to 91 single cells per run using only three corner coordinates to define the sampling grid. Probe touchdown time was optimized to balance analyte desorption and throughput. Small metabolites returned to baseline within 15–30 s, whereas lipids such as PC 34:1 required up to 45 s. Shorter touchdown times increased throughput, while longer times improved metabolome coverage.

Parallel acquisition enabled sequential MS/MS of dozens of targeted phosphatidylcholine species during each 0.57 s Orbitrap full scan. CID and HCD fragmentation generated diagnostic ions for resolving phospholipid isomers and isobars, increasing molecular specificity without compromising duty cycle. Together, automation and parallelized data acquisition provide a streamlined platform for high-throughput single-cell metabolomics with enhanced molecular annotation.

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Targeted NMR-based metabolomics in food science applications

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NMR-based metabolomics hold the power to decode the hidden molecular landscape of food. Data processing pipelines, which effectively handle issues like signal interferences (1), positional deviations (2), and broad background signals (3), are often required to reveal the molecular content from complex NMR data. Here, different pipelines for targeted NMR-based metabolomics were designed for plant-based food matrices not widely studied by NMR before.

First, a pipeline was adapted for pea-based emulsion gels to investigate how transglutaminase and/or ultrasound treatment affects gel breakdown and metabolite release during in vitro digestion (4). Multivariate statistical analysis was performed on aligned and binned spectral data to identify key metabolites for targeted profiling. The results indicated that transglutaminase treatment was associated with lower glucose release during digestion.

Second, a conventional pipeline that comprised ultrafiltration, targeted profiling, and multivariate statistics was employed on different yoghurt analogues based on faba bean and oat (5), results indicating that the bacterial starter culture used for fermentation was a primary driver for the metabolic profile. Furthermore, metabolites like glucose and lactate (but not sucrose and acetate) correlated with sweet- and sour taste, respectively. The designed pipelines provide excellent examples where Targeted NMR-based metabolomics act as the bridge between data, molecular profiles and functional outcomes, highlighting its power in modern food science applications e.g., to map gastrointestinal digestion, study the impact of different treatments, decode sensory attributes, and track fermentation.

AI declarations: OpenAI (chatGPT) was used to make the text flow better and improve readability e.g. The research is the authors' own contribution.

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NMR-Based Quantification of Protein Methylation Uncovers Metabolic Control and Pathological Remodeling

Tobias Huberts, Hansjörg Habisch, Daniel Waltenstorfer, Tobias Eisenberg, Tobias Madl

The accurate quantification of post-translational modifications (PTMs) is imperative for the elucidation of cellular regulation and dysfunction. Protein arginine methylation (ArgMet) and protein lysine methylation (LysMet) represent major subclasses of PTMs that play central roles in diverse (patho)physiological processes.

Herein, a highly robust and versatile nuclear magnetic resonance (NMR)-based methodology is presented, facilitating the concurrent quantification of global ArgMet and LysMet, from a solitary biological sample. The analytical approach requires minimal instrumentation and sample preparation, yet offers high sensitivity and robustness, allowing detection of these PTMs in purified proteins, cells, mammalian tissues and can be combined with NMR-based metabolomics. We demonstrate that these modifications are widespread and dynamically regulated by cellular metabolites and small-molecule inhibitors, and exhibit characteristic alterations in cancer. The method enables comprehensive investigation of methylation pathways and their interaction with related pathways, as well as the identification of relevant biomarkers for diseases and aging.

Therefore, this integrated analytical method provides a powerful tool to investigate the coordinated regulation and biological significance of protein methylation in health and disease.

Metabolome implications of prolonged solid-state fermentation of yellow peas with koji mold (*Aspergillus oryzae* RIB40)

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Solid-state fermentation (SSF) with filamentous fungi offers a promising strategy to modify protein-rich plants, yet the temporal metabolome changes underlying this process remain insufficiently characterized. Here, we investigated metabolome remodeling of yellow peas during SSF with *Aspergillus oryzae* RIB40 under open and sealed conditions over 3, 5, and 7 days using untargeted LC–MS/MS metabolomics. Principal component analysis (PCA) revealed clear differentiation driven by fermentation time, particularly for pea and control mycelial samples grown under open conditions. ANOVA-simultaneous component analysis (ASCA) of pea samples confirmed that all experimental factors and their two-factor interactions significantly contributed to metabolome variation with inoculation explaining the largest effect size (13.6%), followed by oxygen availability (6.6%), and inoculation-oxygen interaction (6.5%). Pairwise PERMANOVA of inoculated pea samples showed that open fermentation induced substantial metabolome remodeling already by day 3 (day 0 vs day 3: $R^2=70.9\%$, $P < 0.05$), with slight additional increases at days 5 and 7. In contrast, sealed fermentation showed a slower and less pronounced early response, followed by more progressive changes at later stages.

Design-specific PCA trajectories demonstrated that inoculated open samples underwent the most pronounced and continuous metabolome transition. PC1 captured progressive depletion of pea substrate compounds and accumulation of fermentation-associated metabolites, whereas PC2 reflected transient accumulation of intermediate metabolites at day 3 followed by further conversion during prolonged fermentation. Molecular networking color-coded by PC1 loadings was used to visualize these trajectories in compound families revealing both modification of native pea metabolites and emergence of fungal secondary metabolites. Differential analysis further showed a strong predominance of upregulated features across all fermentation timepoints, consistent with accumulation of substrate-derived transformation products and fungal metabolites, such as dipeptides, nine small molecular cysteine protease inhibitors (e.g., kojistatin A), and terpenoids (e.g., heptelidic acid). Downregulated features were comparatively limited and included polar carbohydrate-related compounds.

Among food-relevant chemical changes, SSF promoted modification of pea triterpenoid saponins, including depletion of DDMP-conjugated forms and formation of sapogenols, with potential implications for bitterness and nutritional functionality. Fermentation also enriched peas with betaines (trimethylglycine, carnitine, hercynine, stachydrine, and ergothioneine) and polyamines (spermidine and agmatine), metabolites previously associated with flavor formation and health-related functionality. However, prolonged fermentation promoted accumulation of paspaline-type indole diterpene intermediates, suggesting that extended SSF may produce safety-relevant metabolites. Overall, these findings demonstrate that *A. oryzae* RIB40 substantially functionalizes yellow peas. Fermentation time and oxygen availability are key determinants of the balance between desirable food-matrix transformation and secondary metabolite accumulation.

Screening and analysis of off-flavour in potato protein from potato-starch side-stream

Astrid Lund Paulsen, Trine Kastrup Dalsgaard

The demand for alternative proteins has risen due to increasing concerns about the environmental and ethical impacts of animal products, along with projected population growth. Potato protein is a promising alternative protein due to its high-quality amino acid composition and is unique for its high content of the essential amino acid lysine (6.0% of total protein) (1), which is often limiting in other plant-based crops. In addition, potato protein has the advantage of being approved for human consumption, and large quantities are already produced as a side-stream (potato juice) in the potato-starch industry. However, its use for human consumption is limited due to off-flavour.

Off-flavour is a common challenge in many plant-based proteins, caused by compounds co-extracted during protein isolation. Off-flavour formation in the potato starch side-streams is expected to have multiple origins and is thus, by nature, highly diverse. This includes biologically active secondary metabolites produced by the potato, such as triglycoalkaloids (TGAs) and other phenolic compounds. Off-flavour is also often produced by endogenous enzymes in the plant including lipoxygenases (LOX), leading to the formation of volatile secondary compounds that can impact the sensory quality of food (2).

To enable the use of potato proteins for human consumption, the off-flavour challenge must be addressed. This study addresses this challenge in collaboration with Europe's two largest potato-starch industries KMC in Denmark and Avebe in the Netherlands. Potato juice from five different cultivars of industrial relevance is screened and analysed to identify the metabolites that may contribute to undesirable taste, and to obtain knowledge to understand the mechanisms by which they are formed. This will be carried out using various analytical methods, including liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) using targeted and untargeted methods, as well as different extraction methods will be tested.

The data will be analysed using multivariate data analysis (chemometrics), while molecular networking will be used in an attempt to identify additional relevant plant-based flavour metabolites, as the identification of features often serves as a limiting factor when working with plant metabolites, for which comprehensive libraries do not exist.

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Comparative UHPLC-HRMS/MS metabolomics reveals phylogenetic and seasonal patterns of polyphenol diversity across 55 oak species (*Quercus* L.)

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Plant specialized metabolites form a dynamic chemical interface between plants and their environment. In oaks (*Quercus* L.), polyphenols, particularly tannins, contribute to defense against herbivores and other biotic and abiotic stressors [1,2,3]. Their production varies across developmental stages and environmental conditions. Despite their ecological importance, comprehensive metabolomic assessments of polyphenol diversity and seasonal dynamics across phylogenetically diverse oak species remain limited. We applied ultrahigh-performance liquid chromatography coupled with a diode-array detector and high-resolution Orbitrap mass spectrometry (UHPLC-DAD-HRMS/MS)-based metabolomics to characterize foliar polyphenol variation across 55 oak species growing in California and representing a broad range of phylogenetic lineages. We also investigated lineage-specific and seasonal patterns in specialized metabolite composition.

Leaf samples collected throughout the growing season were analyzed by UHPLC-DAD-HRMS/MS, and the resulting metabolomic features were processed using MZmine [4]. Metabolites were annotated based on accurate mass measurements, UV spectra, fragmentation patterns, and comparison with reference compounds. This annotation was followed by compound-specific quantification of the major polyphenol groups. In total, 151 polyphenolic metabolites were detected and characterized. Ellagitannins constituted the structurally most diverse metabolite class, followed by flavonoids and proanthocyanidins. Comparative analyses revealed pronounced differences among sections within the genus *Quercus*, particularly in the abundance of C-glycosidic and glucopyranose-based ellagitannins. Analysis of seasonal metabolomic dynamics revealed a shift from ellagitannin-dominated profiles early in the growing season toward increased accumulation of proanthocyanidins later in the season, highlighting the dynamic nature of oak chemical defenses.

Our results reveal strong phylogenetic structuring and seasonal variation in oak polyphenol metabolomes. This study demonstrates the potential of comparative metabolomics to uncover patterns of chemical diversity and metabolome dynamics across evolutionarily diverse oak species. These findings provide new insights into the ecological and evolutionary roles of plant specialized metabolites.

A Condition-Rich LC–MS Retention Time Library

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Background: Retention time (RT) provides an orthogonal dimension to accurate mass and MS/MS, strengthening compound annotation, enabling better alignment across runs, and improving confidence in library-driven identification. Reliable RT prediction is increasingly important for scalable metabolomics workflows, including quality control, feature prioritization, and reducing ambiguity in isomeric or near-isobaric cases. However, the utility of RT both as a library attribute and as training data for machine-learning models depends critically on the reliability, traceability, and curation of the underlying experimental measurements.

Methods and results: We focused on acquiring RTs with high reliability and accuracy under systematically varied LC conditions. We measured RTs for 2,441 unique compounds across 90 experimental conditions spanning: three temperatures (35/45/55 °C), three pH values (2.5/5.5/7.0), acetonitrile, methanol, and their mixture as the eluent, three gradient profiles, and two C18 columns (Thermo Scientific™ AccuCore™ C18 and Thermo Scientific™ Hypersil GOLD™ C18). Each injection was spiked with approximately 210 compounds. Chromatograms were processed using an in-house workflow built on Thermo Scientific™ Mass Frontier™ algorithms, including FISH identification, component detection, spectra deconvolution, and spectral pattern identification. RTs were post-processed with Python scripts to resolve disputed assignments and flag outliers; questionable cases were manually reviewed and used to iteratively refine the workflow. All workflow was done automatically.

From a theoretical 219,690 peaks, 149,703 (68.1%) passed automated quality criteria; failures were primarily due to low signal intensity, unclear peak shape, or compound - compound interactions. A final targeted manual review highlighted that suspicious RTs could persist for low number of peaks even after complex processing.

Impact: The curated RT database was subsequently used to train and validate a new condition-aware machine learning RT prediction model.

Our analysis highlights the importance of carefully evaluating experimental retention time (RT) data before using it for machine-learning applications. RT values derived from heterogeneous sources, especially when the original chromatograms are unavailable and the data-processing workflow is not fully defined or standardized, can be challenging. Therefore, we recommend a cautious approach to incorporating RT from external or unverified sources, with preference for datasets that include traceable chromatographic evidence and clearly documented processing criteria.

From Thousands of LC-MS features to reproducible biological signals: A Cross-platform Validation and Harmonization of LC-MS Untargeted Metabolomics Data for Precision Health Research

Cecilia Martinez Escobedo, Marina Armeni, Anton Ribbenstedt, Otto Savolainen, Frank Hu Rikard Landberg, Clary Clish, Clemens Wittenbecher

Cross-platform compatibility remains a bottleneck in collaborative metabolomics research. Harmonizing untargeted LC-MS datasets across platforms has the potential of identifying robust, reproducible, and biologically relevant known and unknown LC-MS features. It could also improve compound identification efforts and enable meta-analyses beyond annotated metabolites. Here, we aim to align untargeted LC-MS datasets from several metabolomics platforms.

Plasma samples from 260 participants in the Swedish SIMPLER cohort were analyzed using untargeted reverse-phase LC-MS with positive and negative electrospray ionization at Chalmers Mass Spectrometry Infrastructure (CMSI), the Broad Institute Metabolomics Platform (BIMP), and Metabolon Inc. We matched features across platforms using m/z -based alignment followed by Spearman correlation analysis. We also applied metabCombiner, which aligns features using m/z values and estimated retention-time shifts without requiring bridging sample measurements.

Using mass tolerance ≤ 5 ppm and Spearman correlation $\rho \geq 0.5$ thresholds, alignment between CMSI and BIMP yielded 689 matched features, including 602 features unknown on both platforms, 37 identified only by BIMP, 22 identified only by CMSI, and 28 identified on both platforms. With the same criteria, known Metabolon metabolites matched 347 BIMP features and 173 CMSI features. metabCombiner classified 1078 CMSI-BIMP features as robust matches (score ≥ 0.5) and 600 overlapped with correlation-based matches (55.55%).

Metabolomics measurements in the same samples can support cross-platform matching of unknown LC-MS features despite differences in methods, instruments, and software. Comparison with metabCombiner suggests that same-sample measurements are critical when platforms differ substantially. Applications include annotation transfer from identified metabolites and cross-study validation or meta-analysis of untargeted metabolome-wide association studies.

Development and characterization of a multibarrel probe for nano-DESI-MS

Armin Molanaie, Lucie Davidová, Ingela Lanekoff

Nanospray desorption electrospray ionization (nano-DESI) enables direct sampling and mass spectrometric analysis of molecules from tissues and cells. However, the extraction and ionization efficiency of analytes depend on solvent composition, and a single solvent may not be optimal for all analytes on a tissue. Changing the extraction solvent during analysis can be time-consuming, as it requires replacing the supplied solution and realigning the primary and secondary capillaries. In this work, we explore a multibarrel probe (MBP) as an alternative for solvent delivery in nano-DESI MS. The probe contains 7 independent pressure-driven channels for solvent delivery, allowing solvents or reagents to be introduced through the same primary capillary.

The MBP was fabricated from a multibarrel borosilicate capillary and pulled using a micropipette puller. The probe was pulled and beveled to obtain a tip diameter of approximately 120 μm . Fused-silica capillaries (380 $\times$ 450 μm) were inserted into each individual barrel and fixed using a UV-curable adhesive. For surface sampling, the MBP was used as the primary capillary and positioned together with a secondary capillary to form a liquid bridge at an angle of approximately 95°. The middle channel was continuously operated at a flow rate of 0.5 $\mu\text{L}/\text{min}$ to provide a constant solvent flow and was also the channel through which the spray voltage was applied; the outer channels were controlled independently using a pressure flow controller. The flow was induced using Venturi effect by applying a N_2 gas pressure at 4,500 mbar and a spray voltage of 4 kV. Experiments were conducted on an Orbitrap QE mass spectrometer operated at 120,000 resolution.

The setup was evaluated using spots containing a range of standards, with touchdown measurements of approximately 3 min to compare the effect of different solvents on extraction efficiency. Additionally, tissue sampling was performed on mimetic liver tissue, where the probe was held at a single position for approximately 5 min while the solvent composition was gradually changed, for example from water to IPA. The experiments showed that polar metabolites gave higher signals under aqueous conditions, whereas lipid signals increased with increasing IPA content. Interestingly, the gradient also demonstrated the possibility of sequentially extracting different molecular classes from the same sampling location depending on the solvent composition.

Overall, the MBP provides a flexible platform for controlling solvent composition during nano-DESI sampling. Further work will focus on exploring single-cell analysis and imaging.

Multi-criteria HPLC–HRMS/MS prioritization of CRAM-like features in the fungal exometabolome

Mahsa Norouzi, Anna Rosling, Alexander Craig, Jeffrey Hawkes

Dissolved organic matter (DOM) is a highly complex mixture of organic compounds that plays a central role in aquatic and terrestrial ecosystems, influencing microbial activity, water quality, and long-term carbon storage. A substantial fraction of DOM is considered recalcitrant DOM (RDOM), which resists microbial degradation and can persist for thousands of years in marine systems. An important contributors to RDOM is carboxyl-rich alicyclic molecules (CRAMs); however, their structural definition and origin remain poorly constrained. In environmental metabolomics, high-resolution mass spectrometry (HRMS) is widely used to characterize DOM at the molecular-formula level, but the extreme chemical complexity of DOM, limited structural information, and unresolved isomeric diversity make confident CRAM annotation challenging.

To improve the prioritization of potential CRAM-like molecular features, we developed an untargeted HPLC–HRMS/MS data-processing workflow that integrates multiple orthogonal criteria derived from the literature and experimental observations. Molecular features were evaluated based on: (i) elemental formula assignment within established CRAM-like regions of van Krevelen space, (ii) retention time agreement with in-house CRAM-like model compounds, (iii) tandem MS evidence for sequential CO₂ neutral losses, consistent with multiple carboxyl groups, and (iv) microbial stability after exposure to a microbial community, reflecting the recalcitrant character expected for CRAM-like compounds. This multi-criteria strategy reduces reliance on molecular formula alone and provides a more conservative framework for assigning putative CRAM-like features in complex environmental metabolomics datasets.

We applied this workflow to investigate whether the fungal exometabolome produced by a wood-decay fungus contains persistent CRAM-like molecular features during the transformation of lignin-derived phenolic compounds. The white-rot fungus *Punctularia strigosozonata* was cultured for 26 days in modified Melin–Norkrans (MMN) medium enriched with phenol, vanillic acid, and syringic acid, followed by a one-week microbial degradation phase to assess feature stability. Samples were solid-phase extracted and analyzed by HPLC–HRMS/MS, and raw data were processed in MZmine for feature detection, alignment, and molecular feature table generation. The resulting feature table was then evaluated using the proposed multi-criteria workflow. We report the proportion of molecular features meeting each criterion and assess the extent to which fungal transformation of phenolic substrates generates persistent, carboxyl-rich, CRAM-like signatures. This work demonstrates the potential of exometabolomics-based LC–HRMS/MS workflows to improve the prioritization of structurally ambiguous DOM components and to investigate biological sources of recalcitrant organic matter.

Pushing detection limits in clinical metabolomics: enhanced dynamic range scan mode across conventional and microsampling applications

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Global metabolomics is increasingly applied in clinical contexts, from biomarker discovery and patient stratification to monitoring of microbiome–host interactions and inborn errors of metabolism. However, its clinical translation is constrained by the analytical performance of conventional LC-MS workflows, which struggle to balance sensitivity, dynamic range, and metabolome coverage in complex biological samples. The challenge is most acute for clinically relevant low-abundance compounds, such as microbial metabolites, signaling molecules, hormones, and in microsampling applications, e.g. dried blood spots (DBS), where small volumes push relevant metabolite levels close to the limit of detection. Improving instrument-level sensitivity is therefore a prerequisite for routine, deep clinical metabolomics.

Here, we benchmarked enhanced Dynamic Range (eDR) full-scan acquisition combined with intelligent MS/MS triggering (AcquireX) on a modified Orbitrap-Hybrid-mass-spectrometer (mOH) against the routine QExactive Orbitrap (QE) workflow used in our clinical metabolomics laboratory. The platforms were compared across a hospital-derived pooled plasma reference material (~4,000 patients aged 0–94 years), pooled DBS from healthy volunteers, and patient samples from multiple matrices and pathological states. Methanol extraction and reversed-phase HPLC (Pursuit XRs Diphenyl, 47.5 min gradient) were used in positive ionization mode, with data processed in Compound Discoverer 3.5.

In plasma, eDR clearly extended metabolome and xenobiotic coverage compared with conventional full scan, with mzCloud spectral matches increasing by ~29% and corresponding gains in in-house library annotations. Signal-to-noise (S/N) for low-abundance metabolites improved markedly, for example, serotonin S/N increased from 139 (QE) to 287 (mOH full-scan) and 547 (mOH eDR), while the gut microbial–derived metabolite trimethylamine N-oxide showed a 26% S/N gain with eDR. The number of detected medications more than doubled with eDR (from 8 to 18, including warfarin, metoclopramide, diazepam), highlighting added value for clinical pharmacology and toxicology.

The strongest gains, however, were observed in microsampling. In DBS, the QE detected 310 compounds versus 941 on the mOH, with in-house library matches rising from 36 to 73 and mzCloud matches from 51 to 177. Clinically informative metabolites such as paraxanthine, phosphorylcholine, and cystine were detected exclusively on the mOH, with eDR providing further gains up to 5-fold for cystine, 2.5-fold for serine, and 62% for creatinine, a key kidney function marker. Microbially derived indole-3-acrylic acid and N,N,N-trimethyllysine showed up to 5-fold and 2.6-fold S/N improvements over the routine QE workflow.

Enhanced dynamic range Orbitrap-mass-spectrometry meaningfully extends coverage in standard clinical matrices and truly shines in microsampling, enabling deeper, more sensitive clinical metabolomics analysis.

Accelerating Quantitative Urine Profiling by Low-field NMR Using a Paramagnetic Co-Solute

Jacopo Patrussi, Leonardo Tenori, Alessia Vignoli

NMR-based Metabolomics is traditionally performed using high-field NMR systems (typically 600 MHz), but its high instrument cost and the need for specialized infrastructure limit its translation into clinical practice. Low-field Nuclear Magnetic Resonance (NMR) spectrometers offer an accessible and portable alternative to high-field instruments, enabling applications in diverse settings, including clinical environments. Low-field instruments help address these limitations but suffer from reduced sensitivity and resolution. This is particularly critical in metabolomics, where complex biological matrices produce highly crowded spectra, leading to significant peak overlap and reduced metabolite discrimination (1).

Paramagnetic lanthanide complexes are commonly employed in NMR spectroscopy to enhance spectral performance through relaxation effects (2). The presence of unpaired electrons significantly accelerates nuclear relaxation, resulting in shortened longitudinal relaxation times (T_1). This effect is particularly advantageous in low-field benchtop NMR, where limited sensitivity typically necessitates a high number of scans. By reducing T_1 , shorter scan delays can be applied, enabling faster signal averaging and substantially decreasing overall acquisition times. In this work, we used gadolinium (III) diethylenetriaminepentaacetate (Gd-DTPA) to exploit its paramagnetic properties for urine metabolite analysis.

Urine samples were lyophilized and subsequently resuspended in 90% D_2O and 10% potassium phosphate buffer. The addition of Gd-DTPA as a co-solute was investigated across a concentration gradient to evaluate its impact on relaxation enhancement and peak line broadening. Spectra were acquired at 100 MHz using a WET (Water Suppression Enhanced through T_1 effects) pulse sequence. Preliminary results indicate that Gd-DTPA at 0.5 mM induces significant line broadening, compromising spectral quality. Lower concentrations (0.1–0.3 mM) were therefore explored, with 0.2 mM emerging as the optimal compromise between T_1 shortening and linewidth preservation, while still providing a meaningful sensitivity gain.

The addition of small amounts of paramagnetic Gd-DTPA, readily available as a commercial contrast agent, enables faster and quantitative urine analysis in a cost-effective manner. This approach supports the development of improved low-field NMR workflows for metabolomics as point-of-care diagnostics, extending the analytical capabilities of benchtop systems for clinically relevant biofluids.

Metabolomics of Antarctic Marine Ecosystems: Does Depth Shape Chemical Signatures in DOM and Biomass?

Alexandros Polyzois, Dionysia Rigatou, Eleni Kytinou, Dionysis Raitsos, Nikolas Fokialakis, Triantafyllos Kaloudis

Antarctic marine ecosystems harbor highly dynamic and chemically underexplored microbial communities shaped by extreme environmental gradients. However, the molecular organization of these ecosystems across water-column depth remains poorly characterized. Here, we apply LC–MS/MS-based environmental metabolomics to investigate depth-associated chemical diversity across dissolved organic matter (DOM) and biomass-associated metabolomes collected during the 2nd Greek Expedition to Antarctica, in the marine region surrounding Livingston and Snow Island.

Seawater samples were collected from multiple Antarctic stations across depth gradients (0–50 m), enabling the exploration of metabolomic stratification in relation to ecological and physicochemical variation. DOM fractions were isolated using solid-phase extraction, while parallel biomass sampling enabled integrated characterization of extracellular, intracellular, and dissolved organic matter (DOM)-associated metabolomic profiles. Untargeted high-resolution LC–MS/MS analysis was combined with HPLC chlorophyll profiling and computational metabolomics workflows to characterize ecosystem-scale chemical diversity.

GNPS-based molecular networking, in silico annotation, and comparative metabolomics analyses were employed to investigate molecular family organization and depth-associated chemical patterns. The integration of DOM and biomass metabolomics enabled the exploration of complementary ecological signatures and revealed extensive uncharacterized molecular space distributed across environmental gradients. Particular emphasis was placed on evaluating how depth influences metabolomic composition, potentially reflecting shifts in microbial activity, photochemical processes, and trophic interactions.

Preliminary analyses indicate pronounced depth-dependent metabolomic differentiation between surface and deeper waters, supporting the hypothesis that vertical stratification is a major driver of Antarctic marine chemical organization. The detected molecular diversity highlights the potential of polar ecosystems as reservoirs of unexplored metabolites and chemically mediated ecological interactions.

This work represents the first applications of large-scale LC–MS/MS environmental metabolomics within a Greek Antarctic expedition and demonstrates the value of computational metabolomics for decoding ecosystem-level chemical complexity in extreme marine environments.

A Validated LC-Orbitrap MS Workflow for Wheat Root Exudate Metabolomics

Eka Dian Pusfitasari, Camilla Lind Salomonsen, Josefine Bøgild Krogsgård, Wajeeha Shamsi, Yanyan Zhao, Simona Radutoiu, Marianne Glasius

Root exudates are complex mixtures of metabolites released by plant roots into the surrounding soil environment, where they influence nutrient availability, microbial communities, and belowground biological interactions (1). Comprehensive characterization of these compounds requires analytical workflows that provide broad metabolite coverage together with reliable metabolite identification and quantification.

In this study, an ultra-high-performance liquid chromatography coupled to Orbitrap high-resolution mass spectrometry (LC-Orbitrap MS) workflow was developed and validated for integrated targeted and untargeted metabolomics of wheat (*Triticum aestivum* L.) root exudates (2). Chromatographic conditions, including mobile-phase additives and aqueous mobile-phase composition, together with key data-dependent acquisition (DDA) parameters, were systematically optimized to improve signal intensity and metabolite coverage. The optimized method employed 0.2% acetic acid in Milli-Q water as mobile phase A and acetonitrile as mobile phase B, together with DDA settings of MS1 resolution 120,000, MS2 resolution 15,000, TopN 10, and DeepScan cycle 4, providing a balance between signal intensity, MS/MS coverage, and metabolite identification. Method validation was performed using representative metabolites covering diverse metabolite classes, including amino acids, organic acids, fatty acids, nucleobases, vitamins, and phenolic compounds, such as phenolic acids and flavonoids. The method demonstrated good linearity, sensitivity, accuracy, and within-day and between-day precision for most evaluated compounds. Recovery and matrix effects were compound-dependent and varied between ionization modes. Application of the validated workflow to wheat root exudate samples resulted in the detection of 1034 features in positive ionization mode and 1141 features in negative ionization mode. Metabolite annotation revealed that carboxylic acids and their derivatives dominated the positive ionization dataset, while negative ionization mode provided broader coverage across diverse chemical classes. Targeted analysis further confirmed the presence of amino acids, organic acids, phenolic acids, and flavonoids, including phenylalanine, tryptophan, ferulic acid, succinic acid, coumaric acid, and naringenin.

The developed workflow provides a robust platform for comprehensive metabolomic characterization of wheat root exudates and will facilitate future studies of plant metabolism, rhizosphere chemistry, and plant-microbe interactions.

Key words: wheat root exudates, LC-Orbitrap MS, metabolomics, method validation, targeted metabolomics, untargeted metabolomics, data-dependent acquisition (DDA)

Spatial metabolomics using capillary electrophoresis-mass spectrometry with hydrodynamic injection

Natalia Remper, Ingela Lanekoff

Capillary electrophoresis-mass spectrometry (CE-MS) is a powerful analytical technique for separating and detecting metabolites in chemically complex samples. However, samples for CE-MS are typically homogenized prior to analysis, which results in the loss of spatial information on metabolite localization. To retain the spatial information and still take advantage of CE-MS, we developed surface-sampling CEMS (SS-CE-MS), which enables direct sampling from specific tissue regions while preserving spatial information. In previous work, electrokinetic injection was used for sample transfer into the separation capillary. Here, we present a redesigned SS-CE-MS platform employing hydrodynamic injection to minimize potential sampling biases and improve sampling reproducibility for spatial metabolomics.

The SS-CE-MS platform consisted of fused silica capillaries for analyte extraction and separation, a high-voltage power supply (Spellman SL10) operating at 22 kV, and motorized linear stages (Zaber) for sample positioning during hydrodynamic injection. The system was coupled to an Orbitrap IQ-X Tribrid mass spectrometer (Thermo Fisher Scientific) for high-resolution detection. Surface sampling was achieved by forming a microdroplet between the tissue surface and the probe, allowing localized analyte extraction prior to hydrodynamic injection and electrophoretic separation.

The performance of the platform was evaluated using metabolite standards and mimetic tissue sections. The results demonstrated reproducible separation and relative migration times of metabolites. Within 25 min, a wide range of metabolites extracted directly from the tissue were separated and detected, including amino acids, neurotransmitters, and isomeric compounds such as valine and betaine. The sampling probe was redesigned to achieve a reduced and consistent sampling spot size of 278 μm with 1 % relative standard deviation, compared with previous electrokinetic SS-CE-MS designs. To increase the throughput, multisegment injections directly from different locations on the tissue surface were performed. Consecutive sampling from three distinct locations within a single CE run increased throughput nearly threefold while maintaining consistent analyte separation and migration behavior across all sampled segments.

Overall, the newly developed SS-CE-MS platform enabling hydrodynamic injection was found to provide reproducible, spatially resolved analysis of diverse metabolites with isomeric specificity and improved sampling precision, demonstrating strong potential for spatial metabolomics studies of biological tissues.

A New Pharmacometabolomics Capability at SciLifeLab Drug Discovery and Development: Supporting Decision-Making in Drug Discovery through Metabolomic Profiling

Valentina Ramundi, Ioanna Tsiara, Pawel Baranczewski, Daniel Globisch

In January 2026, a dedicated Metabolomics Capability for drug discovery, Pharmacometabolomics, was established at Uppsala University within the ADME of Therapeutics (ADMEoT) unit of SciLifeLab Drug Discovery and Development (DDD). The capability operates in close collaboration with the Globisch Laboratory, Department of Chemistry for Life Sciences, Uppsala University, led by Professor Daniel Globisch. The primary objective of Pharmacometabolomics is to investigate prototype drug-induced alterations of the metabolome. By integrating metabolomics into the drug discovery process, this approach bridges the gap between genetic variation and phenotypic outcomes, providing a deeper understanding of target engagement and mechanism of action. Metabolomic profiling generates biochemical fingerprints of drug response, supporting both target-based and phenotypic drug discovery strategies, and enabling assessment of safety liabilities and identification of candidate biomarkers for efficacy and toxicity. The approach is further strengthened when combined with proteomics data, offering a multi-layered view of drug action.

Here, we present our Pharmacometabolomics capability, its team, resources, analytical platforms, and the untargeted metabolomics workflow. Based at the Biomedical Centre (BMC) at Uppsala University, our team currently comprises two staff members and supports samples derived from in vitro, animal and human sources, using high-resolution UPLC-MS instrumentation. The workflow comprises four stages: (1) study design, covering sample type and collection strategy; (2) sample preparation and data acquisition, including aliquotation, protein precipitation, centrifugation and lyophilization, followed by LC-MS acquisition and raw data evaluation; (3) data processing, using peak detection, retention-time alignment and isotopic filtering, followed by QC-based sample normalization (QC, RSD, intensity, retention time) and data filtering; and (4) data analysis and interpretation, combining multivariate statistical analysis with metabolite identification against an in-house spectral library, online spectral databases and computational tools, culminating in biological interpretation within relevant metabolic pathways.

Together, these resources allow Pharmacometabolomics to quantify endogenous metabolites, confirm target engagement, and reveal how prototype drugs affect endogenous metabolism and its underlying pathways, thereby supporting decision-making at multiple stages of the drug discovery and development pipeline. By offering this capability to the Swedish and Nordic research communities, we aim to accelerate the translation of academic research into novel therapies, in line with the mission of SciLifeLab DDD as a national drug discovery infrastructure. Currently, the pharmacometabolomics capability is contributing to two exciting projects originating from the SciLifeLab DDD portfolio.

Comparison of plant-based proteins processing on metabolic profiling using an in vitro gut model.

Sara Rocha, Ehsan Shad, Topi Meuronen, Carlos Gómez-Gallego, Marjukka Kolehmainen, Kati Hanhineva

Plant-based protein-rich (PBPR) foods are increasingly popular for health and environmental reasons. However, the understanding of their metabolic impact including the effect on gut microbiota remains understudied. Differentially processed PBPR foods vary in their biochemical composition as using refining technologies may lead to loss of many essential components such as dietary fiber and phytochemicals, when compared to whole-plant-based foods. Such variation can lead to differences in how the gut microbiota interacts with these foods and the resulting health effects, as fermentation by gut microbiota generates an array of bioactive dietary metabolites with potential health implications. Our aim is to understand how Nordic PBPR foods manufactured with different processing technologies can alter the gut microbiota and metabolic profile in an in vitro gut model.

INFOGEST protocol and an in vitro colonic fermentation model to simulate human gastrointestinal conditions was used to characterize the effect on metabolome evoked by 10 PBPR foods, categorized by the used food processing level (whole-plant protein; grinding/mincing; protein isolate/concentrate). Samples were collected at several fermentation time points (0, 1, 2, 4, 6, and 24h) and analyzed by non-targeted LC-MS/MS metabolic profiling. Peak-picking and alignment for spectral data was performed with MS-DIAL software, and data pre-processing and statistical analysis with R with notame package.

Multivariate analysis showed a difference on the metabolic profile of the products made from different plant-based raw materials and different food production techniques along the colonic fermentation. Soy-derived phenolic compounds were prevalent in tofu, made from whole soybeans processed by extensive grinding, while products made via protein isolation were void of them. Minced oats showed to be a notable pool of polyunsaturated fatty acids for gut microbiota digestion, such as linoleic and linolenic acid, compared to products made from whole oat flakes. Tofu, minced oats and minced fava beans displayed a higher abundance of dipeptides and essential amino acids after 24h of colonic fermentation compared to the remaining PBPRs.

These findings suggest that protein source and the processing technology used shape the resulting metabolic profile during colonic fermentation. In particular, mincing or grinding of the food seems to improve the bioaccessibility of PBPR biochemicals to the gut microbiota for digestion. This demonstrates the potential implications the type of processing has for the gut microbiota metabolic impact and provides guidance for product development.

LC-MS and sensory studies on the effect of thermal processing on pungent tasting compounds in black pepper (*Piper nigrum* L.)

Nina Rometsch, John Didzbalis, Matt Harrison, Michael Gigl, Corinna Dawid

Black pepper (*Piper nigrum* L.) is one of the most consumed spices worldwide, with a yearly harvest of 9.21·10⁶ tons in 2024¹. In Germany, the majority of people are consuming cooked dishes at least once a week, but the effect of heating on the compounds responsible for the characteristic pungent taste impression has rarely been investigated so far, despite its influence on the taste and desirability of the final dish². To unravel how the taste of pepper is changed during thermal processing, a combination of classical metabolomics workflows and sensory analysis was applied.

In an initial step, an untargeted ultra-high performance liquid chromatography-time of flight mass spectrometry approach was combined with sensory evaluation to investigate changes in the taste of black pepper caused by heat processing at 70°C, 120°C, 150°C and 180°C. Sensory analysis revealed a loss of pungency in samples heated to a higher temperature (180°C) and differences in the time-resolved perception of pungency via Time-Intensity sensory analysis. The loss of pungency was correlated to the formation of piperine-derived dimers and isomers during the heating, resulting in the degradation of piperine. Six piperine-derived dimers were isolated or synthesized, their structures elucidated, and sensory analysis of the compounds was performed. All processing markers newly identified in the untargeted study and literature-known pungent compounds were included in a LC-MS/MS quantitation method to monitor the effect of thermal processing on black pepper³. To conclude the sensory analysis of the processed pepper samples, the taste thresholds of two of the newly identified dimers as well as of 2 piperine isomers were determined and were up to tenfold higher than that of piperine, providing strong evidence that their formation is crucial to the loss of pungency perceived in heated pepper.

¹ Food and Agriculture Organization of the United Nations, FAOSTAT database

² Bundesministerium für Landwirtschaft, & Ernährung und Heimat (BMLEH) (2025). Deutschland, wie es isst - Der BMLEH-Ernährungsreport 2025.
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Development of a Targeted LC-MS/MS Platform for Broad-Spectrum PFAS Analysis and Exposomics Applications

Jasmin Sebi, Amir Saeid Mohammadi, Hatairad Phetsang

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants occurring across aquatic environments and biological systems. Their broad range of physicochemical properties, particularly from ultra-short- to long-chain PFAS, presents analytical challenges for simultaneous chromatographic separation and sensitive detection. Analytical platforms capable of measuring PFAS across this chemical diversity are important for environmental exposure assessment and can provide targeted exposure data complementary to broader exposomics approaches.

A targeted liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was established and optimized within our mass spectrometry infrastructure, with the aim of expanding the analytical platform to approximately 30 PFAS compounds. Analysis was performed using a Waters ACQUITY UPLC system coupled to a Xevo TQ-S micro triple-quadrupole mass spectrometer. Detection was performed in negative electrospray ionization (ESI[−]) mode using multiple reaction monitoring (MRM), with compound-specific precursor-to-product ion transitions and MS parameters optimized for sensitive and selective detection.

Method performance in an aqueous matrix was evaluated for five PFAS spanning different chain lengths: TFA, PFPrA, PFBA, PFOA and PFOS. Calibration over 2.5–250 µg/L showed good linearity, with coefficients of determination (R^2) ranging from 0.9925 to 0.9989. In no-GAC control samples, TFA, PFPrA and PFBA showed stable concentrations over 24 h, with standard deviations of 0.93–1.29 µg/L at approximately 100 µg/L and coefficients of variation of 0.84–1.14%, reflecting the combined variability of sample preparation and LC–MS/MS analysis.

The method was subsequently applied to a fish matrix, targeting PFNA, PFOS, PFHxS and PFOA. PFAS were extracted using alkaline methanol, followed by clean-up using Oasis[™] WAX for PFAS solid-phase extraction cartridges prior to LC–MS/MS analysis. All four target compounds were detectable using matrix-matched calibration over 0.25–10 ng/g. Comprehensive validation, including evaluation of linearity, recovery, accuracy, precision, matrix effects, and limits of detection and quantification, is ongoing.

Together, these results demonstrate the capability of the established platform for targeted PFAS analysis across environmental and biological matrices. Expansion toward the broader approximately 30-compound PFAS panel and further validation in complex biological matrices are ongoing. By enabling targeted assessment of PFAS exposure across matrices, the platform has the potential to support exposomics studies investigating the relationship between environmental contamination, internal exposure and biological outcomes.

Waters Corporation. Expanding the Range of PFAS in a Single Injection to Include Ultra-Short Chains Using the Atlantis BEH C18 AX Mixed-Mode Column. Waters Application Note. 2023.

Benchmarking Batch Effect Correction Methods for Large-Scale MALDI Mass Spectrometry Imaging of Heterogeneous Prostate Cancer Tissue

Mithlesh P Singh, Maria K Andersen, Ashkan Moradi, Kaisa Irene Lehti, Julia Debik, May-Britt Tessem

Spatial omics technologies have enabled mapping of high-dimensional biomolecular distributions in tissues while preserving anatomical architecture. However, Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) is sensitive to technical variation. Factors such as ion suppression, inhomogeneous matrix crystallization, and variations in sample preparation introduce significant noise [1]. Furthermore, day-to-day fluctuations in instrument performance, and environmental conditions (humidity, temperature), can produce pronounced batch effects [2]. In large-scale studies, these systematic biases can obscure biological signals or produce false-positive correlations, ultimately hindering biomarker discovery. To date, no MSI study has characterized batch effects in cohorts of several hundred samples acquired over multiple years.

In this study, we conducted a systematic evaluation of four different published batch effect correction algorithms [3] (ComBat, WavelCA, NormAE, and Batch Effect Removal Neural Network (BERNN)) using our extensive MALDI-MSI dataset comprising 487 prostate cancer samples from 114 patients collected. The samples were acquired over a three-year period across 125 slides and (~3.1 million spectra at 30 μm resolution, seven technical batches). In the absence of internal quality controls we used 159 biologically relevant masses previously identified in our prostate tissue cohort [2], spanning the 80–1000 m/z range in positive ion mode. We tested each correction algorithm in combination with three normalization frameworks: Total Ion Current, Root Mean Square, and Median normalization. The effectiveness of the batch removal strategies was evaluated visually through Principal Component Analysis (PCA) and Uniform Manifold Approximation and Projection (UMAP) embeddings, and quantified through the Adjusted Mutual Information (AMI) and Adjusted Rand Index (ARI) metrics. To ensure biological integrity, we assessed tissue-type classification performance before and after correction.

Among the four algorithms evaluated, BERNN with Median normalization showed the effective attenuation of batch-driven variance, increasing tissue-type classification performance (Matthews Correlation Coefficient) by 7–8% and producing the improved AMI and ARI scores. However, BERNN incurred substantial computational cost and prolonged training times, indicating a need for scalable correction frameworks adapted to MSI. This study establishes a systematic benchmark of batch correction strategies for large-scale MALDI-MSI.

Decoding emerging microbial lipids and proteins in diet-liver-gut crosstalks

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The gut microbiome produces and modifies a wide range of metabolites, yet microbial lipids remain relatively unexplored. In the present study, we investigated how milk versus yogurt matrices with and without inulin influence microbial metabolism and gut-liver communication during a 6-week controlled dietary intervention in healthy growing male rats ($n = 36$). Animals received one of four diets with incorporated: (1) milk, (2) milk + 5% inulin, (3) yogurt, or (4) yogurt + 5% inulin. Integrated multi-omics profiling, including targeted mass spectrometry-based bile acid measurement, untargeted metabolomics (tandem mass spectrometry), and proteomics, was performed on fecal and liver samples to characterise microbial-modified metabolites and their downstream physiological consequences. We observed modulation of primary and novel microbiome-derived bile acid conjugates, including dipeptide-bile acid conjugates, across diets. In addition, using the GNPS Feature-Based Molecular Networking workflow, we annotated novel conjugated lipid species that had not been previously reported. Multi-Omics Factor Analysis (MOFA) was applied as a general framework for integrating the obtained multi-omics data, which revealed coordinated changes between metabolic features and protein signatures associated with host lipid metabolism and bile acid-linked pathways. Overall, this study provides a comprehensive map of recently discovered microbial-modified lipids and novel bile acid conjugates that are shaped by dairy matrices, linking gut pH, fecal and liver metabolite profiles, and multi-omics protein signatures.

Novelty: These findings reveal previously unrecognised microbial lipid and bile acid modifications and expand our understanding of diet-microbiome-host metabolic interactions.

Exercise as a Modulator of the Human Milk Lipidome

Ankit S. Tanwar, Marta R. Moksnes, Emily R. Ashby, Maëliiss C.C. Lemoine, Guro F. Giskeødegård, Trine Moholdt

Human milk is a compositionally dynamic biofluid whose lipid fraction provides approximately 50% of infant caloric intake and plays critical roles in neonatal neurodevelopment, immune maturation, and long-term metabolic programming. Beyond energy provision, the human milk lipidome comprises structurally diverse lipid classes, including triacylglycerols, phospholipids, sphingolipids, and bioactive lipid mediators with important signaling and metabolic functions. Advances in lipidomic technologies, particularly liquid chromatography-ion mobility-mass spectrometry (LC-IMS-MS), have expanded characterization of the human milk lipidome, revealing considerable inter-individual variability influenced by lactation stage, maternal body composition, diet, and metabolic status. Maternal obesity has been associated with a shift toward saturated fatty acid species at the expense of functional lipids. Emerging evidence further indicates that maternal exercise during lactation may modulate milk lipid composition by remodeling mammary metabolism and increasing the concentration of lipid species linked to reduced adiposity, such as 12,13-diHOME. However, the influence of exercise intensity on the human milk lipidome remains largely uncharacterized.

This randomized crossover study investigated the acute effects of maternal exercise intensity on the human milk lipidome in lactating individuals (n=27) with overweight or obesity (BMI ≥ 25 kg/m²) at 6-8 weeks postpartum. Participants completed three conditions in randomized order: rest, moderate-intensity continuous training (MICT), and high-intensity interval training (HIIT). Milk samples were collected at multiple time points (T0-T4) per condition and analyzed using a targeted lipidomic platform (Complex Lipid Targeted Panel, Metabolon Inc.) capable of quantifying up to 1,100 lipid species across major lipid classes. Following stringent quality control, the effects of condition and time were assessed using linear mixed models and repeated-measures ANOVA simultaneous component analysis (RM-ASCA).

Among 828 lipid species analyzed, 805 showed significant temporal variation, of which 791 remained significant after multiple-testing correction. For time-condition interactions, 12 species differed between MICT and rest, with none surviving correction. In contrast, HIIT versus rest yielded 130 significant species, with one (DAG18:2/20:5) remaining significant after multiple-testing correction. Among 14 lipid classes examined, 12 displayed similar time-condition interaction patterns. Diacylglycerol (DAG) and dihydroceramide (DCER) were notable exceptions, showing persistently elevated levels during rest compared with both exercise conditions across time points. In HIIT condition, triacylglycerol species enriched in saturated fatty acid were elevated, consistent with the overall elevation in total fatty acid content. These findings indicate that acute maternal exercise selectively modulates the human milk lipidome in an intensity-dependent manner.

Securing food supply through early metabolomic plant stress detection in vertical farming

Robin Thür, Lukas Deuring, Wolfgang Sobotzick, Claudia Luksch, Daniel Lanz, Timo D. Stark, Corinna Dawid

Global food security is increasingly challenged by the limited agricultural capacity of land-scarce and import-dependent regions. Countries such as Singapore or the Gulf Cooperation Council (GCC) states rely heavily on food imports, rendering their populations vulnerable to supply chain disruptions^{1,2}. Vertical farming and controlled environment agriculture (CEA) offer promising alternatives by enabling year-round, location-independent crop production with minimal land use. However, realizing this potential requires robust crop health monitoring - a challenge ill-addressed by conventional methods in closed, automated systems.

Plant volatile organic compounds (VOCs) represent a largely untapped resource for non-invasive stress detection. Upon exposure to biotic or abiotic stressors, plants alter their volatile metabolome in characteristic patterns, offering a real-time chemical fingerprint of physiological status^{3,4}. This study investigates VOC-based stress responses of wheat (*Triticum aestivum*) to mechanical damage, drought, heat, and pH stress in a controlled climate chamber.

Volatile sampling was performed by adsorptive enrichment on Tenax® TA sorbent tubes from the chamber atmosphere surrounding stressed and control plants. Samples were analyzed by thermal desorption unit coupled to comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (TDU-GCxGC-ToF-MS), enabling high-resolution separation and identification of complex VOC mixtures. Untargeted data processing and statistical evaluation were conducted using ChromCompare+, with volcano plot analysis applied to identify significantly upregulated features characteristic of each stress condition. Quantification of key stress-indicative volatiles across treatments and replicates is ongoing.

This chemical analytical approach is complemented by a parallel electronic nose (E-nose) system operating within the same climate chamber. While the E-nose provides rapid semiconductor-based signal patterns interpreted through machine learning, the GCxGC-ToF-MS platform delivers the chemical identity and quantitative context necessary to validate and interpret these signals. Together, the dual-platform approach demonstrates that volatile metabolomics can reliably detect and differentiate plant stress states in indoor farming environments.

These findings support the integration of VOC-based monitoring into intelligent CEA systems, contributing to the development of resilient, autonomous food production in regions dependent on stable and sovereign food supply chains.

Chemical Metabolomics – Combining Metabolomics and Chemical Biology Tools for the Discovery of Bioactive Microbiome Metabolites

Torregrosa Chinillach Alejandro, Martin Leo, Lin Weifeng, Theodorou Maria, Kessler Nikolas, Weinkouff Sofie, Barsch Aiko, **De Gobba Cristian**, Lewis Matthew R., Globisch Daniel

The human body is populated by large microbial communities, called microbiota, which profoundly impact the physiology of the human host. While metagenomic analyses are rapidly identifying disease-related microbes, the key metabolites still need to be uncovered as this gut microbial ecosystem produces bioactive metabolites orthogonal to the human metabolism. These molecules can either be toxic or beneficial for the human host. To overcome the inevitable global public health burden posed by multidrug-resistant pathogens, we have investigated microbiome-pathogen co-cultures and human fecal samples using a combination of metabolomics and chemical biology-tools.[1,2] To overcome limitations of metabolite detection in standard global metabolomics analysis, we have developed multifunctional synthetic chemoselective probes with $^{13}\text{C}/^{12}\text{C}$ isotopically labelled analogues that allows for (semi-)quantitative analysis of metabolites in human samples at low concentrations. Coupled to magnetic beads, this method allows the straightforward chemoselective extraction of metabolites from microbial and fecal samples. We have termed this unique method quantitative Quantitative Sensitive CHEmoselective MetAbolomics (quant-SCHEMA). This method has recently been developed for important compound classes of microbial origin such as carbonyls, amines and thiols.[1,2] Our mass spectrometric investigation detected a broad range of human, dietary and bacterial metabolites in fecal and bacterial samples. To directly investigate microbial interactions, we have also developed a platform for the discovery of bioactive metabolites in co-cultures of gut microbes and pathogens.[3] Recently, we have advanced our structure elucidation approaches of the derivatized compounds through DDA-TIMS-MS/MS (Bruker) to significantly improve annotation confidence by comparison with reference CCS values and CCS-prediction in MetaboScape.[4] This additional layer of information led to the annotation of a higher number of metabolites than standard analysis to discover bioactive metabolites.

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Chemical Biology Tools in Metabolomics: Understanding the Gut Microbiome Metabolism in Primary Sclerosing Cholangitis

Alejandro Torregrosa-Chinillach, Peder R. Braadland, Johannes R. Hov, Daniel Globisch

Primary Sclerosing Cholangitis (PSC) is a cholestatic, chronic, liver disease characterized by inflammation of the bile ducts, which leads to impaired bile flow to the gut. It can ultimately result in biliary cirrhosis and liver failure. PSC is considered a rare disorder that affects mainly young males (30-40 years old) in Northern Europe. PSC diagnosis is still challenging as many patients are asymptomatic, and its detection is mostly produced during the evaluation of Inflammatory Bowel Disease (IBD), particularly Ulcerative Colitis (UC) or Crohn's colitis in most patients (60-80%). No pharmacotherapy has been proven to alter the natural course of this disease, leaving liver transplantation as the only temporary curative therapy with a high risk of relapse. Ursodeoxycholic acid (UDCA) is the most studied and commonly used drug. However, its ability to impact long-term outcomes remains uncertain. This therapeutic gap highlights the need for reliable diagnostic and prognostic biomarkers for early detection.[1]

The human gut microbiome is defined as the community of microorganisms (bacteria, viruses, fungi) in our gastrointestinal tract that help to protect from colonization by pathogens, promote the immunoregulation and are involved in the digestion of food. The microbiome is unique for each individual and is influenced by genetics, lifestyle or diet. In addition, microbiome dysbiosis has been linked to the development of several diseases.[5] The gut-liver axis is considered a key factor in the pathogenesis of PSC. Investigation of the co-metabolism represents a huge potential for the discovery of biomarkers. However, the comprehension of the metabolic interaction of the host with the microbiota remains limited. Mass spectrometric metabolomics analysis is the method of choice for analyzing known metabolites. For identification of unknown metabolites, advanced Chemical Biology tools are required but still limited in metabolomics workflows.[2]

Herein, we present a novel Chemical Biology-driven metabolomics workflow for the analysis of human plasma samples comparing the profiles between PSC and healthy controls, as well as PSC patients that underwent liver transplantation. We applied our conjugatome methodology and we identified more than 140 metabolites from microbiome-host co-metabolism. Furthermore, we utilized our innovative chemoselective probe technologies for carbonyl and carboxylate investigations. We observed key metabolic alterations in PSC, which are partly reversible by liver transplantation. Finally, we identified depletion of some bacterial metabolite conjugates linked to PSC, including indoxyl sulfate and β -hydroxybutyric acid. Our results report on new PSC-relevant metabolites as part of the gut-liver axis.

Biocyc Tools For Metabolomic And Multiomic Data Visualization

Suzanne Paley, Miles Trupp, Peter D. Karp

The BioCyc website contains metabolic network data for more than 20,000 microbes and other model organisms, as predicted from their respective genome annotations. 78 of these organisms have benefited from additional manual curation to correct errors and incorporate knowledge from the scientific literature, making them a particularly valuable resource. Including the MetaCyc database which serves as an encyclopedic reference of pan-species metabolic function, BioCyc provides a suite of analysis and visualization tools to aid in interpretation of metabolomic and multiomic data at a variety of scales. We view these tools as a complement to, not a replacement for, more traditional analysis methods. At the lowest scale, the Metabolic Network Explorer explores the metabolic landscape around a single starting metabolite. Users interactively select an input and/or output metabolite to extend backward or forward, iteratively building up a metabolic path. Multiple sets of omics data (metabolomics, transcriptomics, proteomics, reaction flux) can be mapped onto an existing path, or can be used to guide path selection. At an intermediate scale, metabolomics data can be mapped onto pathway diagrams, or onto user-generated collections of pathway diagrams called Pathway Collages. The Cellular Overview Diagram is a zoomable, queryable map of the entire metabolic and transport network. Metabolomics plus transcriptomics and/or proteomics data can be overlaid on this diagram, and multiple timepoints or conditions can be cycled through as an animation, in order to visualize the data in the context of the whole organism. The Omics Dashboard provides an alternative whole-organism view, organizing cellular function into a hierarchical set of systems and subsystems, and presenting omics data as a series of graphs. The user can interactively focus in on particular subsystems of interest as attention is drawn to them. Up to three omics datasets (e.g. metabolomics plus transcriptomics and proteomics) can be displayed simultaneously, each optionally with multiple timepoints or conditions.

Towards a standardized pipeline for genome-scale metabolic model contextualization

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Genome-scale metabolic models (GSMM) enable multiomic data integration based on mechanistic principles and have emerged as a powerful tool in the study of disease mechanisms (1). A barrier to the more widespread use of GSMM is the lack of a standardized pipeline extending across contexts and data types. Here we assemble a pipeline to contextualize GSMM for MASLD patients using transcriptomic data from the MAFALDA cohort (2), considering various existing approaches for data pre-processing, model construction, and structural comparisons of models across patient groups. We outline the conditions under which different methods are applicable and describe ways to detect and control noise throughout the model generation process.

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Investigating the Metabolic Impact of Perfluorooctanoic acid (PFOA) on in-vitro Human CD4+ Regulatory T cell Differentiation by Carbon Labelled 1,2 ¹³C-Glucose using Optimized LC-MS Platform and Lipidomics

Ziyu Yang, Roosa Kattelus, Matilda Kråkström, Alex Dickens, Matej Orešič, Riitta Lahesmaa

Regulatory CD4+T cells, as the master of immunosuppression, have been reported to conduct their functions relying on fatty acid oxidation and OXPHOS during differentiation. On the other hand, the altered lipid profiles have been demonstrated based on lipidomic analysis and genome-scale metabolic modeling (GSMM) in our previous study [1].

The pentose phosphate pathway (PPP) supports nucleotide synthesis and redox balance and may be disrupted by PFAS, which are linked to impaired lipid biosynthesis and enhanced glycolysis, potentially via NRF2 regulation [2,3].

In this study, the most accurate tracer for tracing glycolysis and PPP pathways, positionally labeled 1,2 ¹³C-glucose [4], was applied to investigate the flux from glycolysis and PPP pathways separately in iTregs polarized from cord blood-derived naïve CD4+T cells under PFOA exposure.

According to the results, CD25 expression increased under PFOA exposure, while Foxp3 levels showed a dose-dependent response, indicating complex effects on Treg differentiation. Stable isotope tracing with 1,2-¹³C-glucose revealed broad metabolic perturbations, including altered glycolytic and tricarboxylic acid (TCA) cycle flux. The pentose phosphate pathway (PPP) intermediate 6-phosphogluconate was reduced at higher PFOA levels, and citrate flux was also downregulated, indicating potential carbon redistribution toward lipid synthesis and itaconate production. Lipidomic profiling further demonstrated pronounced lipid disturbances under high PFOA exposure.

CD25 and Foxp3 were analyzed by flow cytometry (Fortessa, FlowJo). Polar metabolites were quantified using a 3NPH-derivatized optimized LC-MS platform, and lipids by reverse-phase LC-MS. Data were processed with SCIEX OS and MZmine4.

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Retention Time Prediction by Learning Thermodynamic Parameters with Graph Neural Networks

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Retention time (RT) in liquid chromatography (LC) is increasingly important for metabolomics and small-molecule workflows, where RT provides an orthogonal method to mass spectrometry, supports targeted method development, and reduces compound identification time. However, RT is highly sensitive to experimental conditions, limiting transferability of experimentally acquired RTs between laboratories and methods. Machine-learning (ML) RT prediction is an alternative, but many ML models share a similar limitation: they predict RT only for experimental conditions identical to training, ignoring system parameters such as dead volume and gradient delay time^{1, 2}.

We present a new ML architecture for predicting retention time (RT), trained and validated on a large dataset generated in a Thermo Fisher Scientific laboratory in Vilnius, Lithuania. The dataset includes 2 293 compounds measured under 90 different experimental conditions, covering different temperatures and pH levels. Acetonitrile and methanol including three gradient profiles were used as mobile phase, including two C18 columns (Thermo Scientific™ Accucore™ C18 and Thermo Scientific™ Hypersil GOLD™ C18). Chromatograms were processed using an in-house automated workflow based on Mass Frontier; extracted RT values were quality-checked and used to train and validate the model.

The ML model is based on a Graph Attention Network (GAN) using molecular structures as graph inputs, augmented with molecular descriptors, column parameters, and experimental-condition vectors. The key novelty is that the GAN does not learn RT directly. Instead, it predicts parameters of a chromatographic model as hidden features. These predicted parameters are then used within the chromatographic model to compute RT, and RT errors are backpropagated through the full pipeline to optimize the ML model parameters.

This strategy makes the predictor condition-aware, enabling extrapolation beyond the training settings: RT can be generated for any gradient program, including non-linear, for any column length and diameter, mobile-phase flow, dead volume, and gradient delay time, as well as for any temperature within the trained range, while maintaining compatibility with the pre-trained columns, pH, and eluent systems.

Across validation experiments, the approach reaches an MAE of 0.93 min, delivering state-of-the-art-level accuracy while generalizing across gradients and system parameters rather than being limited to a single fixed method.

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Ion-to-Image (i2i) v2: An improved mass spectrometry image analysis tool

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Mass spectrometry imaging (MSI) enables non-targeted visualization of molecular distributions across a sample surface. Translating these full mass spectra acquired in each image pixel from raw data to gain insightful biological information requires efficient data management and processing pipelines. However, this is generally hampered by limited compatibility among specialized software tools, making it difficult for researchers across disciplines to effectively analyze complex datasets. Therefore, there is a critical need to develop a unified computational environment that can simultaneously manage the massive dimensionality of MSI datasets and make state-of-the-art analytical tools (e.g., deep learning) accessible to researchers beyond computer science. We have previously reported ion-to-image (i2i), a MATLAB-based tool for ion-image generation and data processing for nanospray desorption electrospray ionization (nano-DESI) MSI. In this work, we present further enhancements to the i2i. In addition to the existing targeted and untargeted analysis capabilities, our new version supports widely used data formats, such as mzML, imzML, (MSI) RAW, and (MATLAB) MAT, for a streamlined workflow. Additionally, we have incorporated a tool to enhance ion image visualization, through super resolution MSI using an Efficient Sub-Pixel Convolutional Neural Network (ESPCN), co-alignment of MSI data, microscopy, and other images, using a DL-based image registration feature that utilizes uniform manifold approximation and projection (UMAP), and unsupervised semantic segmentation of MSI images, by compressing data dimensionality for based on unique chemical signatures. Finally, it also features our previously reported spatial similarity network (SSN) analysis for enhanced annotation of isomers and isobars through their spatial distribution. In summary, by consolidating these advanced deep learning and spatial analysis tools into a single open-source platform, this enhanced framework empowers researchers to efficiently translate high-dimensional raw MSI data into actionable biological insights.

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High-resolution nano-DESI mass spectrometry imaging to investigate metabolic alterations in the plaque and peri-plaque regions in Alzheimer's disease

Connie Petroeschevsky, Gijs Kooij, Ingela Lanekoff

Alzheimer's disease is the most common form of dementia, a disease that affects more than 12 million people in Europe alone (1). The lack of effective disease-modifying treatments for Alzheimer's is partly due to an incomplete understanding of disease processes. It is thought that early metabolic changes in neuronal tissue, particularly altered lipid metabolism, may help drive the formation of amyloid- β deposits and contribute to early neurotoxicity (2). Mass spectrometry imaging (MSI) is a powerful technique for visualizing metabolic changes that occur in spatially distinct patterns across a tissue section. In Alzheimer's disease, MALDI-MSI has enabled visualization of changes in lipid metabolism within and around plaque deposits (3). Nano-DESI is an ambient MSI technique that uses a capillary liquid bridge to locally extract analytes from the tissue surface, followed by electrospray ionization mass spectrometry analysis. Nano-DESI is well suited for quantitative MSI through the inclusion of internal standards in the extraction solvent. As such, nano-DESI is a powerful technique for revealing molecular distributions that is complementary to other MSI methods. Here we used a modified pneumatically assisted nano-DESI setup coupled to a Q-Exactive Orbitrap high-resolution mass spectrometer to image human postmortem brain tissue from Alzheimer's disease cases and age-matched unaffected controls with a spatial resolution of less than 20 μm . Tissues were imaged in both positive and negative ion modes and the extraction solvent composition was optimized for increased molecular coverage. We show metabolic alterations in plaque and peri-plaque regions, which provides a complementary dataset to immunohistology studies and improves understanding of the metabolic changes associated with amyloid- β deposits in human Alzheimer's disease.

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Hypothesis-driven spatial metabolomics: an open-source toolkit for multimodal interrogation of bioactive lipid pathways

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Spatial metabolomics is used to localize bioactive metabolites within tissue. Untargeted methods generate large datasets comprising thousands of putatively annotated features including hypothesis-relevant metabolites and off-target pathways. This increases data, analysis and interpretation complexity. Additionally, untargeted imaging methods alone cannot reliably detect and resolve low abundant lipid mediator isomers (e.g., oxylipins) at biologically relevant concentrations.

We present a targeted, hypothesis-driven, spatial metabolomics workflow. Here a panel of biologically relevant and analytically validated metabolites is determined by LC-MS/MS and then mapped by desorption electrospray ionization-multiple reaction monitoring (DESI-MRM). LC-MS/MS screening enables generation of selected metabolite panels (compatible with high-sensitivity MRM-imaging) that span dysregulated metabolic pathways with isomeric resolution. This targeted spatial metabolomics approach extends to post-DESI imaging, with targeted spatial transcriptomics and immunohistochemistry. Allowing targeted metabolomics data to be overlaid with selected anatomical regions, cell types and enzymes. The result is fewer, but more tractable measurements that are directly interpretable against the study design.

To support this workflow, we have developed four open-source tools.

MRManalyzeR (github.com/MJS-708/MRManalyzeR) processes targeted LC-MS/MS data (S/N and blank filtering, batch correction, PCA/volcano/heatmap HTML reports), and used to assess MRM transition fidelity, resolve isomers, and inform target selection for spatial assays.

quantMSImageR (github.com/MJS-708/quantMSImageR) processes DESI-MRM images, including hot/cold spot removal, normalization, calibration-based quantification, region-of-interest (ROI) integration, and HTML reporting of ion images, metabolite ratios, and inter-metabolite correlations.

tissUUmeps helpers (github.com/BIIFSweden/CraigWheelock2024-1) provides interactive, browser-based visualization integrating MRM-based imaging data with co-registered H&E and spatial transcriptomics; yielding easily shared outputs for non-specialist collaborators to interpret directly.

corrMSI (github.com/MJS-708/corrMSI) enables ROI-resolved comparisons and bivariate spatial autocorrelation (e.g., Moran statistics) across processed spatial datasets. This includes gaussian decay from cell types, niches, or ROIs for regional comparisons and inter-modality correlation (e.g., metabolite vs ROI or metabolite vs cell type) to determine spatial regulation between sample groups (e.g., case/control).

To demonstrate utility of these tools, we present data from studies in allergic mouse airways, where a selected 10-analyte oxylipin panel was detected with DESI-MRM and spatial transcriptomics. The analyzed data show that cyclooxygenase-derived oxylipins, notably PGE₂, localized to airway regions and spatially correlated with recruited immune-cell populations.

This work shows the advantages of targeted multimodal spatial analyses: small per-sample data; rapid spatial metabolomics acquisitions enabled by LC-MS/MS screening (~2h, for 10 Hz on <10 analytes) for larger studies; and simplified data analysis that focuses expertise on biologically anchored target selection and experimental design – yielding highly interpretable readouts for non-specialists in interdisciplinary projects.

Urinary metabolomics revealed diet-specific responsiveness of the Dietary Metabotype Score in adults with overweight and obesity undergoing a controlled nutritional intervention

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Objective assessment of dietary intake remains a major challenge in nutritional research, as traditional self-reported methods are prone to bias. Urinary nuclear magnetic resonance (NMR) metabolomics represents a robust analytical approach for capturing food-related biomarker responses. Leveraging this capability, the MetaboTracker software (Bruker, Germany) was developed and validated in healthy adults to quantify dietary pattern quality from urinary ^1H -NMR data. Its primary output, the Dietary Metabotype Score (DMS), is a composite metric that evaluates the overall urinary spectral fingerprint of individuals based on their adherence to the World Health Organization (WHO) dietary guidelines.

In this study, we evaluated the performance and responsiveness of DMS within a randomized controlled dietary intervention in adults with overweight and obesity, designed to assess the effects of personalized dietary strategies according to insulin-resistance phenotype.

Pooled 24-hour urine samples from 188 participants were analysed at baseline and after a 12-week intervention with either a low-fat high-protein, high fibre (LFHP) diet or a high-monounsaturated-fatty acid (HMUFA) diet. Urinary ^1H -NMR spectra were analyzed using MetaboTracker software (Bruker, Germany). The metabolomics-derived DMS was complemented by 18 diet-responsive metabolites linked to six food-group categories. Different statistical models were used to assess temporal changes in DMS and individual biomarkers, accounting for inter-individual variability and clinical covariates.

The DMS showed a significant increase following the LFHP intervention ($p=0.0078$), with higher scores reflecting greater adherence to WHO healthy eating guidelines, whereas no significant change was observed for HMUFA, underscoring the diet-specific sensitivity of urine-based metabolic fingerprints. In the LFHP arm, several biomarkers of food intake contributed to this response, including plant-associated metabolites such as trigonelline and 4-hydroxyhippuric acid, alongside carnitine-related metabolites linked to protein intake. In contrast, HMUFA-associated changes were modest and did not remain significant after FDR correction, consistent with the limited representation of lipid quality in urinary metabolome. Insulin-resistance phenotype did not significantly modify the DMS scores, indicating that the observed effects primarily reflect dietary exposure rather than metabolic health status.

Overall, this study demonstrated that the DMS tool, based on ^1H -NMR urinary metabolomics, appeared effective in capturing diet-induced metabolic shifts driven by changes in plant-based and protein-related food intake, while also delineating methodological boundaries for diets affecting metabolic pathways not well represented in urine. Together, these findings point toward a future role for metabolomics-derived composite scores in enhancing objectivity and robustness of dietary intake assessment beyond self-reported tools.

P101

Process-resolved LC–MS metabolomics reveals chemical remodeling in an industrial multi-botanical beverage during processing and storage

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Industrial processing and storage can substantially reshape the chemical composition of plant-based beverages, but their metabolomic evolution across complete production workflows remains poorly characterized. In this study, LC–MS/MS-based untargeted metabolomics was applied to characterize the process-resolved chemical remodeling of an industrial multi-botanical beverage formulated with jasmine tea, lemon verbena, and elderflower. Fifteen workflow points were sampled across raw-material infusion, industrial formulation and thermal processing, and one month of bottled storage.

In total, 1865 LC–MS features were detected, of which 943 were annotated. Feature-based molecular networking revealed 611 nodes and 106 molecular clusters, reflecting the high chemical diversity of the beverage matrix. Multivariate analysis showed that raw-material infusions had distinct metabolomic fingerprints, whereas processing integrated these chemically diverse botanical inputs into a more unified matrix. Sucrose addition, acidification, and tea incorporation generated workflow-specific molecular signatures associated with Brix, viscosity, pH, conductivity, color, and antioxidant capacity. During storage, the global metabolome remained relatively stable, but time-series clustering revealed delayed compound-specific changes, including furanic compound accumulation and changes in selected phenolic and tea-derived metabolites.

Integrated marker prioritization identified workflow-sensitive metabolites associated with raw-material identity, processing effects, and storage-related remodeling. Candidate markers, including selected terpenoid-, flavonoid-, coumarin-, and furanic-related compounds, were linked to flavor-associated profiles, antioxidant-related properties, and product stability. Overall, this study demonstrates the value of process-resolved untargeted metabolomics for linking industrial processing, storage chemistry, and quality-relevant molecular markers in complex botanical beverages.

Non-targeted lipidomic profiling reveals distinct molecular signatures in inflammatory bowel disease: discovery and validation in two inception cohorts

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Minimally invasive biomarkers that can reliably distinguish adults with inflammatory bowel disease (IBD) from symptomatic controls are still lacking. We investigated whether serum lipidomics can provide a reproducible diagnostic signature for IBD and how such a signature performs in relation to relevant inflammatory markers. Untargeted lipidomic profiling was conducted on serum from the Swedish SIC-IBD inception cohort, comprising adults referred for suspected IBD, symptomatic controls, as well as healthy controls. Candidate diagnostic lipids were identified using supervised machine-learning models in the discovery cohort and then evaluated in the independent, population-based Norwegian IBSEN III inception cohort of treatment-naïve adults. We examined the diagnostic performance of the lipidomic signature alone and in combination with high-sensitivity C-reactive protein (hs-CRP) and fecal calprotectin (FCP). The discovery cohort included 96 IBD patients, 66 non-IBD symptomatic controls, and 48 healthy controls, whereas the validation cohort comprised 349 patients with IBD and 198 symptomatic controls. A serum signature of the two top lipids, lactosyl ceramide (d18:1/16:0) and phosphatidylcholine (O-44:5), showed the best diagnostic performance in the discovery cohort (0.77 95% CI 0.69-0.79). When applied to the validation cohort, the lipid signature yielded an AUC of 0.77 (95% CI 0.73-0.81). Adding this lipid pair to hs-CRP significantly enhanced discrimination between IBD and symptomatic controls (AUC 0.79, 95% CI 0.75-0.83) compared with hs-CRP alone (AUC 0.68, 95% CI 0.64-0.73; $P < 0.0001$). Among patients with available stool samples, combining FCP with hs-CRP and the lipid signature numerically improved diagnostic accuracy (AUC 0.89, 95% CI 0.86-0.92) compared with FCP alone (AUC 0.86, 95% CI 0.83-0.90). We identified and externally validated a serum lipidomic signature that improves the diagnostic prediction of IBD when combined with hs-CRP. Although the blood-based model of hs-CRP, lactosyl ceramide (d18:1/16:0), and phosphatidylcholine (O-44:5) did not outperform FCP alone, it approached its diagnostic performance and further enhanced accuracy when integrated with FCP. These findings support added value these lipids and may offer insights into lipid-related pathways underlying IBD pathogenesis.

Individual Urinary Metabolic Phenotypes at High and Low Magnetic Field: A Dual-Field ^1H NMR Study

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The existence of a stable, individual-specific urinary metabolic phenotype in healthy humans is well established. ^1H NMR spectroscopy of longitudinally collected urine samples allows near-perfect discrimination of individuals, revealing an invariant metabolic fingerprint persisting over years to decades and exhibiting allostatic resilience under pathophysiological stress^{1–3}. This body of work has relied exclusively on high-field instruments operating at 600 MHz, which, despite superior sensitivity and resolution, carry cost, infrastructure, and portability constraints limiting translation to clinical and point-of-care settings. This study investigates, for the first time, whether the individual urinary metabolic phenotype is detectable and discriminable at 100 MHz, by directly comparing spectral profiles acquired in parallel at both field strengths on urine samples from the same longitudinal cohort. Morning fasting urine samples were collected from subjects participating in previous collections^{1–3} using the same standardized pre-analytical protocol. For benchtop analysis, samples underwent freeze-drying prior to reconstitution. High-field spectra were acquired on a Bruker spectrometer (Bruker BioSpin GmbH, Ettlingen, Germany) operating at 600.13 MHz, equipped with a 5 mm PATXI ^1H - ^{13}C - ^{15}N and ^2H -decoupling probe, z-axis gradient coil, automatic tuning–matching (ATM), and refrigerated sample changer (SampleJet, Bruker BioSpin), calibrated daily following strict standard operating procedures. One-dimensional NOESYGPPR1D and JRES experiments were acquired for profiling and signal assignment. Benchtop spectra at 100 MHz were collected using a comparable solvent-suppressed 1D ^1H protocol. Spectral bucketing, normalization, and multivariate statistical analysis — including PCA, canonical analysis, and k-NN classification with test set validation — were applied consistently across both field strengths. Preliminary analysis suggests that individual-specific metabolic fingerprints remain detectable at 100 MHz, with classification accuracies to be formally assessed against the near-perfect discrimination previously reported at 600 MHz^{2,3}. Establishing how well the low-field fingerprint preserves the discriminative power of its high-field counterpart has direct implications for the democratization of NMR-based metabolic phenotyping and its deployment in decentralized and resource-limited healthcare settings.

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Non-invasive assessment of the skin barrier following treatment in persons with diabetes and xerotic feet

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Foot ulcers are a common complication in persons with diabetes, affecting 4-10% of this population and contributing to substantial morbidity through infection and potential amputation (1). To reduce foot xerosis and prevent wound formation, topical formulations are recommended. Although changes in the biophysical state of the skin barrier are sometimes investigated in clinical studies, for example by corneometry (skin hydration) or transepidermal water loss (TEWL), changes in its molecular composition are rarely assessed. This gap limits mechanistic understanding of skin care efficacy and highlights the need for more advanced noninvasive biophysical measurements combined with molecular profiling of the skin barrier. We investigated if noninvasive methods for assessing skin barrier function may support more targeted and personalized skin care treatment strategies. In a two-armed double blind randomized controlled trial (RCT), xerotic feet of persons with diabetes were treated with a cream containing a humectant (Oviderm or Canoderm) on one foot, and humectant-free cream (Decubal) on the other (2). At baseline and after four weeks, three subsequent tape strips were collected from foot skin for metabolomic, lipidomic and proteomic analyses. In addition, electrical impedance spectroscopy (EIS) and TEWL were measured at baseline and after two and four weeks. The primary molecular classes expected to be detected include ceramides, sterols and free fatty acids, as well as natural moisturizing factors (e.g., free amino acids and derivatives thereof) and proteins such as filaggrin, proteolytic and glycolytic enzymes. In addition to extraction of these molecular classes, total protein quantification using a BCA assay will be performed to normalize the omics datasets and minimize systematic bias arising from uneven amounts of tape-stripped material. The generated data will be used to assess whether distinct molecular patterns are associated with different severities of skin dryness and how these patterns change over time in response to skin care treatment. Multiomic profiling of dry skin, together with the assessment of treatment-induced molecular changes, will advance our understanding of the molecular mechanisms underlying skin barrier function. Ultimately, this approach may clarify how molecular features correlate with macroscopic barrier properties, as probed by EIS and TEWL, and support the development of noninvasive methods for assessing skin barrier integrity, as well as evidence-based skin care regimens to prevent dry and impaired skin.

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P105

Faecal metabolomics to detect metabolic variability in children with cancer

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The gut microbiome acts as a modulator of cancer biology, treatment response, and recovery, but systematic studies in pediatric oncology remain scarce. Here, we present preliminary metabolomics data from stool samples collected from children diagnosed with cancer through the Norwegian Childhood Cancer Biobank and healthy children from the microTromsø cohort. We analyzed samples from 63 pediatric cancer patients and 118 controls aged 0–18 years. Targeted LC-MS metabolomics was used to quantify short-chain fatty acids and related organic acids. In addition, an untargeted LC-MS pilot study was performed to explore broader metabolic differences between groups.

Targeted analysis showed strong age-related variation in fecal short-chain fatty acid and organic acid profiles, consistent with maturation of gut microbial metabolism during childhood. No statistically significant differences in individual targeted metabolites were detected between cancer patients and controls after age stratification. However, cancer patients showed greater inter-individual variability in metabolite profiles, suggesting metabolic heterogeneity or dysregulation at diagnosis. In the untargeted pilot analysis, 671 metabolic features were detected, and principal component analysis indicated separation between cancer patients and controls. A total of 301 features differed between groups in unpaired testing.

These findings suggest that pediatric cancer may be associated less with uniform shifts in short-chain fatty acids and more with broader disruption of the fecal metabolome. Ongoing work will expand the dataset, integrate metabolomics with shotgun metagenomics.

From Discovery to Quantification: Metabolomics Services at Chalmers Mass Spectrometry Infrastructure

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Untargeted and targeted metabolomics provide complementary strategies for investigating metabolism in biological research. Untargeted methodologies offer broad metabolic coverage and discovery potential but can present challenges related to metabolite identification, relative quantification, and comparability between studies. Targeted approaches focus on predefined metabolites and provide robust quantitative measurements through standardized workflows, facilitating comparison across samples, studies, and time points, although with a narrower analytical scope¹.

Chalmers Mass Spectrometry Infrastructure (CMSI) provides services ranging from discovery-oriented untargeted profiling to targeted quantitative metabolomics. These capabilities enable researchers to select the most appropriate strategy or combine approaches, for example by progressing from discovery to quantitative follow-up.

The CMSI targeted metabolomics portfolio includes custom assays as well as standardized panel-based analysis using the Biocrates MxP® Quant 1000 assay². The latter provides broad coverage of predefined metabolites across multiple biochemical classes, supporting a wide range of biomedical applications. The assay can be applied to a range of biological matrices, including plasma, serum, urine, and fecal samples. Standardized sample preparation and rigorous quality-control procedures are incorporated throughout the analytical workflow.

The combined service portfolio supports exploratory profiling, hypothesis-driven studies, quantitative follow-up, and longitudinal or multicenter projects. CMSI supports researchers throughout the project lifecycle, including study design, sample management, mass spectrometry analysis, data processing, quality assessment, statistical analysis, and biological interpretation. Together, these capabilities enable researchers to apply metabolomics in the way that best addresses their research questions.

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Lipidomic Responses to Triclosan Highlight Bacterial Membrane Metabolism as a Therapeutic Target

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The development of resistance to current antibiotics is a major public health concern. Multidrug resistance is especially problematic in Gram-negative bacteria from nosocomial infections, such as *Escherichia coli*. Resistance in virulent bacteria with potential use in bioterrorism is also alarming, highlighting the urgent need for new treatments. One promising strategy targets the membrane lipid metabolism, essential for cellular integrity. Inhibiting enzymes in this pathway offers therapeutic potential. Fatty acid synthase-II (FAS-II) and more specifically the FabZ and FabI enzymes are of particular interest because these enzymes are absent or minimally expressed in humans, and are present in target bacteria. Before testing new FAS-II inhibitors developed by the AGIR team, *E. coli* and *Staphylococcus aureus* were treated with triclosan, a FabI inhibitor known to disrupt bacterial membranes, followed by lipidomic analysis (1).

These two bacteria were selected due to the differences in membrane composition between Gram-positive and Gram-negative bacteria. While *E. coli* is primarily composed of phosphatidylethanolamine (75%), this metabolite is absent in *S. aureus*, whose membrane is structured with some diacylglycerol derivatives, also absent in *E. coli*. However, they share common components such as cardiolipin and phosphatidylglycerol.

Colonies of *E. coli* and *S. aureus* were cultured. Then triclosan was added at 0.25× MIC or 0.5× MIC, or no triclosan for the controls. Bacterial pellets were resuspended in solvents (Folch method), then cell lysis was performed by freeze–thaw cycles or by homogenization. After centrifugation, lipid extracts were dried and resuspended in an injection solvent. Samples were analyzed by LC-MS/MS.

The results we obtained are consistent with the description of the lipids composing the membrane of the two bacterial species. Moreover, we did observe an inhibition of FabI in these two species whose lipidomic profiles differ from each other. A dose effect of the triclosan was observed. For *E. coli*, with increasing dose of triclosan, levels of Fatty Acid Esters of Hydroxy Fatty Acids (FAHFAs) with three double bonds increased whereas levels of FAHFAs with one double bond decreased. In addition, phosphoethanolamines (PE), with only even number of carbons, have increased levels too. For *S. aureus*, with increasing dose of triclosan, levels of phosphoglycerides (PG) and lysophosphoglycerides (LPG) decreased. Results revealed changes in lipid profiles when triclosan was added, bacteria-specific changes.

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Chiral derivatization reagents for multimodal LC-MS and fluorescence profiling of trace d-amino acids

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Background: d-amino acids (d-AAs) are no longer considered merely bacterial components, but are now recognized as vital mammalian signaling molecules and emerging biomarkers for severe pathologies, including neurodegenerative disorders or carcinoma. However, translating d-AA profiling into routine clinical diagnostics is hindered by crucial analytical challenges. d-AAs occur in complex biological matrices at trace concentrations, often orders of magnitude lower than their l-forms. Furthermore, their high polarity and identical physicochemical properties to their l-forms prevent direct separation on standard achiral stationary phases. While pre-column derivatization utilizing chiral reagents allows for diastereomer resolution, conventional commercial tools (e.g., Marfey's reagent, FLEC) exhibit poor electrospray ionization (ESI) efficiency, rendering them inadequate for highly sensitive LC-MS trace analysis.

Methods: To address the critical lack of sensitivity, we designed a novel class of multimodal chiral derivatization reagents based on isoquinoline, β -carboline, and carbazole scaffolds. These structures were engineered for high proton affinity and intrinsic native fluorescence. Following targeted *N*- and *O*-alkylation, the carboxylic acid intermediates were converted into reactive *N*-hydroxysuccinimide (NHS) esters using *N,N'*-disuccinimidyl carbonate (DSC). Strict anhydrous conditions were maintained to prevent hydrolysis, balancing long-term reagent stability with rapid derivatization kinetics toward target amino groups. For the planned application of this platform to murine glioblastoma models, the analytical workflow will directly target the quantification of free d-amino acids. This crucial testing phase ensures accurate biomarker profiling across complex biological matrices, specifically utilizing brain tissue, plasma, and urine.

Results: The target enantiomerically pure NHS-ester reagents were successfully synthesized and structurally confirmed. Upon reaction with model d/l-amino acid mixtures, they rapidly formed stable diastereomers. Chromatographic evaluation demonstrated excellent baseline enantioseparation utilizing standard achiral stationary phases (C18 and PFP). Crucially, the rationally incorporated heterocyclic cores functioned as robust ionization enhancers in positive ESI-MS mode, generating strong $[M+H]^+$ signals. This specific structural advantage directly enabled the achievement of picomolar limits of detection.

Conclusions: The developed platform addresses the critical limitations of current commercial tools by offering a truly multimodal detection strategy. Seamlessly integrating routine fluorescence screening with ultra-sensitive LC-MS quantification, this methodology provides a highly accessible and cost-effective workflow that eliminates the reliance on expensive chiral chromatography. This enhanced capacity is essential for the accurate profiling of d-amino acids in complex biological matrices, ultimately facilitating their validation as reliable clinical biomarkers for severe pathologies such as glioblastoma.

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P109

Improving Metabolic Flux Analysis for Isotopologue Ratio Measurements using a Novel Dual Ionization GC-EI&CI-TOFMS including a newly developed CI Source

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Accurate analysis of isotopologue-ratios of stable isotope labelled metabolites is essential for tracer experiments and metabolic flux calculations, which are key techniques for pathway analysis in metabolic engineering. Mass spectrometry (MS)-based methods offer high accuracy in isotopologue distribution analysis.

Gas chromatography (GC) coupled to MS is valuable for flux analysis but often lacks intact metabolite carbon backbone information due to extensive fragmentation in electron ionization (EI). To overcome this limitation, a novel chemical ionization (CI) source was developed and operated in parallel with a conventional EI source on a GC-TOFMS system. This setup enables simultaneous acquisition of intact molecular ions (via CI) and positional isotope information (via EI) in a single GC run, reducing analysis time and resource demand.

Isotopologue abundances of central carbon metabolites, including amino and organic acids, sugars, and sugar phosphates, were measured using a validated automated two-step derivatization method. Various CI reagent gases were tested with automated switching, and isotopologue intensities were corrected for natural isotope contributions. As reference materials are still lacking, trueness was evaluated using in vivo labelled ethanolic yeast extract. Combination of CI- and EI-data enhanced compound identification confidence in retrospective untargeted analysis by improving molecular and positional information of the labelled atoms.

P110

Expanding plasma metabolome coverage with enhanced dynamic range and iterative MS/MS acquisition on a modified Orbitrap hybrid mass spectrometer

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Untargeted plasma metabolomics is challenged by the broad concentration range of endogenous metabolites, where high-abundance ions can dominate MS1 precursor selection and reduce MS/MS sampling of lower-abundance species. We evaluated enhanced dynamic range (eDR) MS1 acquisition and iterative MS/MS acquisition as a workflow to expand plasma metabolome coverage using NIST SRM 1950 pooled human plasma.

Plasma proteins were precipitated with cold methanol, and extracts were analyzed by reversed-phase LC-HRMS using C18 chromatography on a modified hybrid high-resolution mass spectrometer. Conventional and eDR acquisition were compared across normalized plasma-equivalent loads (0.58, 1.44 and 4.33 μ L on column), with eDR acquisition segmenting the m/z 67-1000 scan range into 16 windows at 120,000 resolution setting operated in a positive ionization mode. AcquireX Deep Scan DDA used blank-derived exclusion, three MS1 reference/replicate injections and six or seven sequential iterative MS2 identification injections acquired at 30,000 resolution setting. MS/MS quality was further evaluated at different isolation widths. Data was processed in Compound Discoverer 3.5 and FreeStyle 1.8 software, using compound-level feature grouping, blank filtering and quality filters based on reproducibility, formula assignment, chromatographic peak quality, mzCloud spectral-library score and MS/MS purity.

eDR increased quality-filtered MS1 compound entities at every plasma-equivalent load, with the largest gain at 4.33 μ L: 6,619 entities versus 1,591 by conventional full scan (+316%). Strictly filtered mzCloud MS/MS matches also increased at this load, from 509 to 859 (+69%). Representative cortisone traces showed improved signal-to-noise through ion injection-time redistribution, with the largest injection-time gain at the lowest load, while phenylalanine chromatograms (representing one of the dominating species) confirmed that increased plasma loading preserved peak shape and retention time. Together, eDR and optimized DDA iterative MS/MS acquisition improved the efficient use of MS/MS duty cycle and enabled higher plasma-equivalent loading to translate into broader, quality-filtered metabolite coverage and more confident putative annotation.

P111

Pushing the Limits of Structural Lipidomics: Enhanced Sensitivity and MS³-Driven Annotation on a Modified Orbitrap Tribrid Mass Spectrometer

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Advances in lipidomic analysis require mass spectrometry platforms that deliver high sensitivity, rapid acquisition, and efficient parallel use of multiple mass analyzers. A modified Orbitrap-based tribrid mass spectrometer was evaluated to meet these demands by improving sensitivity, enabling faster polarity switching, and enhancing parallelization between the Orbitrap and linear ion trap. Bovine liver lipid extracts were used to evaluate the analytical performance of the modified MS, and plant leaf extracts were analyzed with the optimized workflow. Samples were spiked with a deuterium-labeled internal standard mixture to improve relative quantification. The lipids were separated using C30-based reversed-phase chromatography, coupled with polarity-switching high-resolution MS1 precursor analyses and data-dependent fragmentation, combining higher-energy C-trap dissociation (HCD) and collision-induced dissociation (CID) with ultraviolet photodissociation (UVPD) for lipid fragmentation up to the MSⁿ level. Preliminary data show that the limit of detection (LOD) ranged from low picomoles to high femtomoles for all detected internal standards. For instance, 15:0-18:1(d7) phosphatidylcholine (PC) showed an LOD of 25 femtomole on-column in the positive mode, while 15:0-18:1(d7) phosphatidylinositol (PI) showed an LOD of 4 picomole on-column in negative mode. Enhanced parallel operation of the two detectors, together with rapid polarity switching, improved our duty-cycle efficiency and markedly increased data density while maintaining quantitative accuracy in lipid measurements. Our MS method used a 1.5-second cycle time and employed HCD and CID-based MS2 fragmentation, with triggered CID-based MS3 fragmentation to resolve coeluting isomeric triacylglycerols, and neutral-loss-triggered UVPD-based MS³ to determine regioisomers of PC species. This approach, combined with manual data curation, enabled the determination of the exact fatty acyl composition and sn-positions in selected PC species, thereby increasing the number of unique lipid IDs. IRMPD-based photofragmentation of standard lipids was tested out. Finally, using commercially available software, we assessed the lipidome of plant extracts and identified over 1000 unique lipids in the dataset.